

Converting intelligence into wisdom

DOES Britain use its intellectual resources wisely? There can be little doubt that the British education system has proved itself competent in the past at identifying and nourishing the high flier, from primary school through to university. Even in this time of egalitarianism there still seems a recognition of the need to sustain the outstanding student who may contribute something of exceptional value to society in the future. Twenty years of education can turn out people whose intellectual skills have been polished and sharpened to a high degree. But do we provide the right environment, as a nation, to capitalise for the next forty years on these skills; not just for the benefit of an organisation but also so that the individual may continue to feel a sense of fulfilment from being intellectually extended?

There is a trivial sense in which every educational system is immensely wasteful, and must be so to be effective. The vast majority of what is learnt in school or university is never specifically going to be used later in life but can only at best be described as intellectual compost—material which helps to explain ‘the way things are’ and which may illuminate future thinking in an undefinable way. But there is a far less trivial, and far more nation-specific way in which intellectual resources may be used or squandered. If the structure of a society is so heavily compartmentalised that the only way ahead is to become a specialist, and progressively to acquire greater expertise in a narrower discipline, then there is a small chance that intelligence will lead to wisdom. If there is an element of cross-movement open, at all levels, there is at least the possibility that every year will bring sounder judgement and more job-satisfaction.

Britain is indubitably a compartmentalised society: civil servants, academics, lawyers, doctors, politicians, industrialists and many others—they all pursue their own separate and highly specialised paths, defending themselves against outsiders and looking askance on those few who choose to step off a well-defined ladder.

The civil service is as good, or bad, an example as any. Competition for entry into the service is fierce; very intelligent people enter the service in their twenties. Whatever the label they carry—administrative, scientific and so on—they can be reasonably sure of a lifetime of employment, and indeed very few ever think of changing career. Certainly during that lifetime for many there will be varied work to do, but it will all be within the framework of serving the state. Even

within this, the largest compartment of them all, there are any number of sub-compartments. A scientist who, at the age of 40, feels he has run out of steam is not going to find the administrative or executive branches waiting with open arms for him, regardless of the undoubted variety of experience he might bring to a new job. Not, it must be admitted, that civil servants show all that much interest in changing their environment; a recent scheme to encourage temporary moves between the service and industry and academe, after two or three years of very modest success, is now in abeyance.

Because the civil service has built up great bodies of specialist expertise (some excellent, some not) within its walls, it feels relatively little need to share its problems with others or to draw in the outsider for a period to give a new perspective. This then leaves academics, for instance, ill-informed on many questions of broad national or international interest and inclined to withdraw even further into their laboratories, common rooms and learned societies. Not of course, that academics can be depicted as entirely innocent of their own compartmentalisations, both to keep the outside world at bay and to maintain fine distinctions between disciplines.

The end result of all this is by no means that Britain lacks experts in almost every subject under the sun, but that broader perception is often lacking. And surely scientists, as well as anyone, know how important a multidisciplinary approach is in the advancement of knowledge and understanding.

Of course, mobility must not be preached for its own sake. There are many who are at their happiest and most productive when pursuing their own specialisation, and these would profit little, and be much irritated by an ethos in which they were viewed with suspicion for sticking to what they do most effectively. On the other hand there are equally many who would profit greatly from the chance to diversify their career.

No single grand gesture will shake Britain out of its present over-specialised character. Rather a variety of smaller things have to be done: attitudes must change, terms of employment must be revised, even the penalty that the mobile suffer in their pension must be looked at. And there certainly ought to be more serious discussions about developing new institutions which could effectively span the public and private sector, looking at major policy problems but from an outsider's point of view. □



US boosts basic science, but overall R & D drops

Defence, energy and space head for large increases in basic research funding in President Carter's 1980 budget.

David Dickson reports

"THE budget for fiscal year 1980 is lean and austere". The words that President Carter chose to open his budget message to Congress on Monday apply directly to proposed funding levels for research and development—but in a time of financial conservatism basic science has done relatively well, being scheduled for a real growth of 20% above the expected level of inflation.

The overall size of the R&D budget reflects the President's choice of reduced public spending as a major weapon to fight inflation. Thus total R & D obligations are planned to rise only 4.1% to \$30.6 billion, the smallest increase in the R & D budget since the Nixon era.

Given this general constraint, the main issue that has occupied government agencies in preparing their budget requests has been to decide how the money should be distributed along

the R & D spectrum. And it seems to have been generally accepted that optimal long-term results can be achieved by shifting support for 'development' projects back to the basic research end (a trend initiated last year, though dealt with unsympathetically by Congress).

The net result of this is a projected increase of 9% in the funds available for the support of basic science, about 2% above the expected level of inflation, with increases of twice this amount for some agencies, such as the departments of energy and defence.

Those projects to have suffered are the ones which the administration feels have reached a stage at which they could be taken up in other ways—if at all. There will, for example, be a reduction in support for coal conversion technologies and hydroelectric and solar heating R & D, although more

money for basic research in solar energy.

One agency to have done well in the budget review is the Department of Defense, which accounts for about 45% of federal support for R & D. The department is scheduled to receive an increase of 7% in its total R & D funds to \$13.8 billion, and within this an increase in basic research of 17% from \$373 to \$436 million.

The department's research money, whose growth reflects a general growth in defence spending that characterises the whole budget, will be used to support projects such as the MX intercontinental ballistic missile and new research on very high speed integrated circuits. There will also be more defence money spent in university research departments.

An even greater increase—18.8%—is scheduled for basic research supported by the National Aeronautics and Space Administration. However, here the figure is slightly deceptive, since much of this will be required to

Space scientists disappointed: The budget for the National Aeronautics and Space Administration does not contain any major new mission starts. It comes as a disappointment to space scientists, who had hoped that at least some of their proposals might have escaped the cuts. Top of the list of projects which will not receive funding are plans to send an orbiting imaging radar to Venus—particularly important in view of the failure of some of the equipment on board the current Pioneer missions—and plans for a gamma ray observatory.

Other casualties include a national oceanographic satellite system, and plans to place a block order for a number of multi-mission satellites (mms). These will now be ordered individually, which could increase the costs of proposed British mms. Although these and other new projects were included in a \$4.9 billion request which NASA put to the Office of Management and Budget, they were all discounted to ensure that general budget constraints and increased shuttle costs did not cut into existing programmes.

10% more for university research: The total federal R&D outlays to universities and colleges is planned to increase by 10% in 1980, considerably above the overall increase in R&D funding. The largest increase will be in research funds from the Department of Defense, scheduled to grow on campuses by 13% to a total of \$359 million. Other major increases are planned for DHEW (11.9%) and the National Science Foundation (12.1%). In the proposed budget for the National Institutes of Health, funds for research projects awarded on a competitive basis are planned to fall from \$494 million to \$279 million, while non-competitive funds are scheduled to increase by approximately the same amount.

Boost for nuclear physics: After several years of relative neglect, there is some good news for nuclear physics, and government officials say that the increased support should continue "for some years to come". Funds have been

made available in the Department of Energy's budget for a major heavy-ion facility at Michigan State University; an energy doubling electron beam recirculator at the Bates Linear Accelerator of the Massachusetts Institute of Technology; and an experiment staging area at the Anderson Meson Facility of the Los Alamos Scientific Laboratory. The expansion in nuclear physics has been partly made possible by the near completion of the Cornell storage ring, funded by the National Science Foundation. Construction of the Michigan facility is to be transferred from the NSF to the Department of Energy, although the foundation will continue to cover research costs.

Research on environmental hazards: As a reflection of the growing importance of scientific findings in the control and regulations of environmental hazards, the administration is proposing to make a major increase in the research budget of the Environmental Protection Agency. Obligations for R&D will increase from \$400 million to \$436 million, with a 16% increase in longer term research.

NSF instrumentation grants up 50%: The National Science Foundation is planning a 50% increase in its allocation of funds for research instrumentation and equipment, a source of growing concern in recent years and one of the areas in which universities and research institutions have been hit hardest by inflation. Announcing this increase, NSF director Dr Richard C. Atkinson said: "There is no question that we have been lagging behind in equipping our laboratories". In many Western European countries, the proportion of the research budget devoted to instrumentation was almost three times as high as in the US. The increase will raise the funds for instrumentation from \$54.4 million in the current fiscal year to \$81.6 million. The extra money is contained in a 12.4% increase in NSF funds for research in physiology and cellular and molecular biology, and a 15.4% increase for environmental biology.



Carter: fighting inflation with spending cuts support projects that have already been initiated—such as the Jupiter Galileo mission—but are now moving into an expensive phase.

Another burden on NASA's budget is the increased costs of the Space Shuttle, for which the agency is already having to approach Congress for an extra \$185 million as a result of delays caused by engine test failures. The net result is that although there will be a 20% increase in NASA's sup-

port for space science and applications, there is no provision for any new programmes.

At the Department of Energy, which is having to bear much of the cutback in federal spending, there is said to have been considerable dispute over which end of the R & D spectrum should bear the brunt of the cuts. In the end demonstration projects were the ones to suffer. Within an overall R & D budget that will remain virtually constant in 1980 at \$4.6 billion, there will be a 17% increase in support for basic science, sufficient to ensure continued support of high energy physics at current levels, as well as a boost for nuclear physics and solar energy research.

As far as the National Science Foundation is concerned, the administration has asked for a relatively modest overall increase in funding of 8.4%, bringing the agency's budget to \$11.006 billion. Basic research is scheduled for an 11.8% increase, with large increases in chemistry, the earth sciences and environmental biology, and particular emphasis on upgrading equipment. Unlike previous years, no

great increase is suggested for NSF's applied science activities. The budget for "problem-focused research applications" is to fall by 11.6%.

Projected figures for the Department of Health, Education and Welfare are a somewhat unreliable guide to what will actually be spent, since traditionally they are raised substantially by Congress. With this in mind, the administration has requested a budget for the National Institutes of Health of \$1.4 billion, less than 1% higher than the current year.

Commenting on the overall budget figures, Dr Frank Press, Director of the Office of Science and Technology, said that "in general we feel that R & D has received good treatment. And when you add the basic research initiatives, we feel this is a very strong research budget."

Dr Press said that President Carter's initiative in increasing funds for basic research had become a model for other countries spurring similar increases, for example, in France, Britain, and West Germany. "It is nice to see that the US research dollar has leverage of this kind", Dr Press said. □

Sun shines on solar energy: Research on solar energy, a proposed cut in which last year brought a storm of protest from the environmental movement, is planned to receive an increase in funding of 24%. Longer term solar-related technology development and applied research will grow by 40% in 1980. In particular, the Department of Energy's budget request includes funds for the construction of a new 300-acre research facility at the Solar Energy Research Institute in Golden, Colorado.

Administration tries again on competitive agricultural research grants: The US Department of Agriculture is once more proposing a major increase in funding for competitive research grants, a proposal which has come under heavy fire in Congress for the last two years, during which such increase have been considerably cut back. In its proposed 1980 budget, the President has asked for a total of \$30 million, two-thirds of this for basic research on the efficiency of food production, and the rest for the improvement of human nutrition. One major initiative in agricultural research is a major inter-agency effort to assess the value of space remote sensing data in obtaining early estimates of the effect of natural disasters on crop conditions, and improving world-wide agricultural production forecasting. Total funding for these projects, which will involve the departments of Agriculture, Commerce and Interior, as well as NASA and AID, is estimated to increase from \$18 million in 1979 to \$29 million in 1980.

No funds for Clinch River or nuclear reprocessing: The Administration is sticking to its previous position on two hot political issues in the nuclear field by requesting no funds for either continuation of the liquid metal fast breeder reactor at Clinch River in Tennessee or for nuclear fuel reprocessing. Although \$504 million is being requested for LMFBR research and development, indicating a continued commitment to the fast breeder, the administration repeats its position that the proposed

Clinch River demonstration project "is both premature and uneconomical".

Research on nuclear fuel reprocessing is proposed for termination in 1980, in line with President Carter's non-proliferation policy. In its place, emphasis will be placed on "once through" nuclear fuel cycle that will be used in conjunction with improved and more efficient light water nuclear power plants. Research funds for the Department of Energy provide additional support for the examination of alternative geological sites for a deep nuclear waste depository, and for developing new waste forms that could be used for the disposal of high level defense wastes. President Carter has also announced that he plans to introduce a "spent nuclear fuel Act" which will authorise the department to accept spent nuclear fuel for storage and disposal in return for a one-time charge.

Major effort in microelectronics: Three federal agencies—the Department of Defense, the National Science Foundation, and the National Bureau of Standards—are proposing to carry out complementary efforts in the field of microelectronics and submicron science and technology in 1980, involving a total budget of \$41 million. At the NSF, efforts will be concentrated on fundamental research into the properties of microstructures, aimed at supporting a number of applications in electronics. The foundation will also provide training for scientists in this field, where a shortage of graduates is claimed by some to be holding back the efforts of private industry. The Defense Department is planning a major expansion of its research into very high speed integrated circuits, despite a major split in the defence industry over whether or not the technology already exists to meet the department's needs.

President halts child research building: Permission to construct a \$37 million child health research facility at the National Institutes of Health has been withdrawn. It was considered low priority in a time of budget stringencies.

New US agency to focus research aspect of development aid

David Dickson reports from Washington on President Carter's proposal for a foundation for International Technological Cooperation

ONE of the relatively few new initiatives in the budget recommendations submitted by President Carter to the US Congress last Monday was the proposal to establish, under the foreign aid appropriations, a Foundation for International Technological Cooperation (FITC).

The foundation is intended to act as the focus for the research and development component of the US development assistance effort. It is presently being conceived as one element in a proposed international development cooperation administration, which would also include existing bodies such as the peace corps and the Agency for International Development (AID).

Funding for the FITC would be about \$100 million for the first year of full operation, beginning 1 October 1979, although only a relatively small amount of this—about \$25 million—would be new money, the majority of support resulting from the transfer of research projects currently carried out by AID. Eventually it is hoped that the foundations will have an annual budget of three to four hundred million dollars, the same order of magnitude as the National Science Foundation.

Plans for the foundation already figure prominently in the US national paper for the United Nations Conference on Science and Technology for Development (UNCSTD). However, criticisms of various aspects, such as the degree of autonomy which FITC will—or should—be able to exercise from existing aid programmes, and from overt US interests, indicate that the administration may face an uphill task in getting its plans accepted.

The proposed foundation was announced last March in a speech by President Carter in Caracas, Venezuela. It is largely the brain child of Dr Frank Press, director for the President's Office of science and technology policy, and reflects the same type of approach to R&D planning that he has injected into areas of domestic policy.

A planning office, reporting directly both to Dr Press and to Mr John G. Gilligan, administrator of AID, was established last summer. It is directed by Dr Ralph Schmuckler, previously dean of international studies and programmes at Michigan State University, and chairman of AID's research advisory board.

According to Dr Schmuckler, the

foundation will have two main objectives, first, to help strengthen the scientific and technical problem-solving capacities of developing nations; and second to focus attention of US scientific resources on problems of concern to developing countries.

Although precise details are yet to be worked out, FITC is expected to operate by identifying various "problem-areas"—in contrast to the country-by-country programming of bilateral aid programmes—and then to work out which are the crucial problems in these areas.

"The programming process will be a bit like a funnel. At the top we will have all types of state-of-the-art analyses, with participation by agencies already involved, by developing country representatives, and by scientists. We would then move from a broad sweep to an increasingly narrow focus on particularly important goals that could be realised over a period of five to ten years", Dr Schmuckler said.

To illustrate the type of research that might result from this process, he cites work being supported by AID on the possible production of a vaccine against malaria, "A good example of an analysed, research problem dealt with initially in a very low-cost way, but resulting in some scientific breakthroughs that offer the possibility of a solution to a major health problem".

Besides research itself, however, an equally important part of the foundation's activities will involve helping to build research infrastructures in developing countries—for example by

assisting in the training of scientists and technologists through courses and seminars, and occasionally providing short-term financial support for local research initiatives.

"We aim to spend the majority of our money in the developing countries themselves—a rough estimate is 75%", says Dr Schmuckler. "The rest will go to US universities and research institutions, but only to support work which is directly tied to activities overseas".

And to ensure what he calls a "real live audience" for the foundation's efforts, roughly one-third of FITC's advisory council—which will be responsible for recommending areas for study to the foundation's director—will come from developing countries.

Legislation to set up the foundation is soon to be presented to Congress as part of an amendment to existing foreign aid legislation. However, there are already signs that various criticisms of the current plan could lead to a tough political battle.

One criticism, for example, is that the foundation will be so closely linked to existing agencies such as AID that it will not have sufficient autonomy to apply an objective analysis to third world research needs (although others point out that it may be necessary to emphasise US interests to get the FITC accepted by Congress).

"The basic ideas is a very good one—but I am not very happy about the way that things are working out, particularly since the foundation seems to be becoming a subsidiary of AID almost as soon as it is born", says Professor Michael J. Moravcsik, professor of physics at the University of Oregon, and an active member of the recently-formed Council on Science and Technology for Development.

Professor Moravcsik and others are now putting their support behind an alternative proposal suggested by Senator Adlai Stevenson for a foundation for international scientific and technological cooperation, an agency similar in concept to the FITC, but having the power of autonomy enjoyed by the National Science Foundation.

A bill proposing such a FISTC is to be introduced into Congress by Senator Stevenson within the next few weeks. Attractive as the greater autonomy may be to some, however, its chances of success against the administration's FITC proposal, in a Congress increasingly concerned about public accountability for the use of federal funds, remains uncertain. □



Above: protection against malaria. The disease disables 30–40% of the working population where the disease is rife. Research into a vaccine is "a good example" of the work FITC might undertake.

Cadmium puts UK village at risk

CADMIUM, a light metal mined with zinc, has been discovered in abnormally high amounts in stream sediment in the village of Shipham, the site of a zinc mine abandoned two hundred years ago. The Department of the Environment (DOE) has warned the villagers not to eat local produce and not to smoke and has initiated a £70,000 investigation to study the effects on health.

Levels of cadmium found in the soil varied from 11 ppm to 998 ppm. The accepted safe level is less than 1 ppm. Serious cadmium poisoning occurred in Japan from 1962 to 1968 caused by levels of cadmium in rice paddies of 1–50 ppm although there were other contributing factors in Japan that are not present in Shipham—such as dissolved cadmium in water.

Shipham water has been tested and is “absolutely safe” said Mr Denis Howell (a minister in the Department of Environment). “There is cause for concern but not for alarm. It is in nobody’s interest to exaggerate the alarm” said Howell. Local residents have known for years that vegetables grow yellow in certain locations, and there is a local ailment—called “Shipham tummy”—which could be a sign of chronic cadmium exposure. Long term effects are poorly known but symptoms of cadmium poisoning are diarrhoea, vomiting, stomach pains and choking.

The high concentrations were discovered in the course of an Imperial College geochemical reconnaissance of England and Wales begun in 1967. The study took 50,000 stream sediments

from tributary drainage over the whole country during a ten week period in 1969. A complementary sampling of soil and rock was undertaken in Shipham to test for the presence of elements in areas of poor drainage. The high cadmium count found in this survey was followed up by sampling garden soil in conjunction with researchers from the Westminster Hospital Medical School in Spring 1978. These results were released to the DOE last August.

Dr Ian Thornton, Geochemistry Department at Imperial College and participant in the original study says that Shipham is probably unique. Calamine, ZnCO_3 , was mined in Shipham whereas in other mines it was galena, zinc sulphide.

Joe Schwartz

Biochemist challenges redundancy

AN industrial tribunal currently underway in Manchester raises new questions on the power of granting agencies to make scientists redundant. Dr David Pillinger, FRIC, 39, a biochemist employed at the Christie Hospital in Withington near Manchester, is the first senior staff member of the National Health Service to be made redundant since 1948. Pillinger’s case, brought to the tribunal with the aid of ASTMS, squarely confronts a complicated legal issue affecting thousands of scientific workers in the UK; who is their employer? Is it the agency that provides the money or the institution that does the hiring?

Pillinger was hired in 1962 by the Manchester Area Health Authority to do cancer research at the Christie Hospital. At that time there was no special category for research workers and Pillinger was engaged on the same basis as the clinical biochemists and physicians, with a permanent NHS contract, the funding coming from the British Empire Cancer Campaign. In this arrangement the Health Authority provides a permanent contract even though it does not provide the funds, with the understanding that the contract will be underwritten by the Authority should outside money dry up.

In 1972, Professor L. G. Lajtha, Director of Research in the Christie Hospital’s Patterson Laboratory, attempted to bring all research workers under MRC short term contracts of three to five year duration. Pillinger refused to sign such a contract but eventually was persuaded in 1973 to take a post of unlimited duration on a block grant, a form of funding that

covers several different projects. This was a hybrid contract in which the employer was still the Health Authority but the money and pay scales were set by the MRC. Says Pillinger, “There is no doubt, nor has it ever been questioned, that my employer is the Area Health Authority.”

Nevertheless in June 1978, Pillinger was told by Lajtha that he was to be made redundant. In a letter from the MRC Lajtha was told that there was to be no reduction in funding for Pillinger’s chemotherapy project but that the MRC did not feel “that Dr Pillinger should continue to be supported even though a senior scientist is needed”. Pillinger pressed Lajtha for an explanation and in notes submitted to the tribunal quotes Lajtha as saying, “I doubt if we will ever be told, since the MRC is an autonomous body. It does not have to give account to anybody for any of the decisions they take.”

The Christie Hospital administration is supporting Pillinger. Mr D. C. Critchley, former Sector Administrator, was called by Pillinger’s counsel, Ms Janet Smith, to testify last Monday. Lajtha, however, is critical of the hospital administration. Calling Pillinger’s case a “purely administrative problem” Lajtha feels that Pillinger’s original contract was a case of “administrative irresponsibility” and that the old management should have made it clear that a scientist’s employment must depend on the ebb and flow of grant money. Lajtha says that the MRC project grant renewal did not permit him to employ a Senior Grade Scientist but only a Grade 2 Scientist (equivalent

to a Lecturer). When asked why he did not make representations on Pillinger’s behalf to the MRC Lajtha said that he had already presented the case to the MRC and that they were “fully in the picture”.

Behind the official language of dismissal and tribunal, however, lies a story of research conflict and scientific disagreement. In 1969 Pillinger returned from a two year stint at Columbia University in New York where he worked with Ernest Borek on tRNA modification by methylation. Pillinger began work on this line at the Christie Hospital and produced 12 publications in three years. In 1972 this project was terminated by the MRC. Lajtha requested that Pillinger start work on a model of cancer cell proliferation in which tissues were hypothesised to produce proliferation inhibitors. Pillinger’s job was to isolate the putative inhibitors called chalones. The technique used cell extracts in water and the fraction of MW of around 1000 was observed to inhibit cell division. Pillinger was critical of this work and thinks that the results were artefacts. (The cell division assay used radioactive thymidine as a marker and Pillinger felt that the cell extract fraction found to inhibit division probably contains thymidine so that a decrease in thymidine uptake was an artefact of the preparation.) In 1974 Pillinger transferred to other work on cancer chemotherapy with Brian Fox, Head of Experimental Chemotherapy. It was on this project grant that the MRC found that Pillinger’s services were no longer necessary.

Joe Schwartz

UN desert unit to concentrate on national projects

DISILLUSION with transnational programmes to halt the growth of the world's deserts is causing the United Nations Environment Programme (UNEP) to turn to specific national projects, the new director of UNEP's desert unit said last week.

The director, Jens Høgel, said that the criticisms of Professor Mohamed Kassas (reported in *Nature* last week, p167) were "a long step ahead" from the original political naïvety of the 1977 United Nations Conference on Desertification (UNCOD), which proposed six giant transnational projects based more on scientific optimism than political reality. Professor Kassas was in fact a major force behind UNCOD.

UNCOD passed the six projects on to UNEP, which then had the task of implementing them. Details of the projects—which include matters such as the management of the massive North East African fossil water aquifer—are still being drawn up, but, says Høgel, "it is better to put our eggs in national baskets".

One such plan is for the establishment of new industries in arid zones to attract populations back on to desert margins. These would bring water with them and help to stabilise desert movement. Nine countries—Afghanistan, Algeria, India, Iraq, Mali, Mexico, Peru, Spain and Tanzania—are submitting project proposals to a meeting which will take place in Moscow from 5–8 February. The USSR will provide the necessary funding.

Nevertheless national progress "is still going a bit too slow". And while many rich northern states "have indicated very clearly that they are interested" in supporting UNEP's desert work, no money will be forthcoming from them until specific projects are agreed.

"But there is no reason for despair" says Høgel. It took 30 years from the recognition of the problems of development around the time of the second world war before effective action began

to be possible through the United Nations Development Programme (UNDP) and so on. "We have just recognised desertification (at UNCOD) in October 1977. But it won't take three decades now; we have UNEP, a new will to succeed, and a tremendous pool of international expertise to call on."

Nevertheless there is a great deal of inertia to overcome. The independent Club du Sahel, funded mostly by the US and France, was created in 1975, "but when I left the Sahel four months ago there still wasn't any action".

Høgel is adamant that the UN organisations have not been "sluggish and inefficient" as charged by Professor Kassas. "We are at the top of the scale compared with most bilateral agencies, and what is more you can't lose the United Nation's goodwill." On the other hand, Høgel can see bilateral agencies imperialistically forcing their solutions onto the recipient countries. "This leads to reticence, the resulting institutions won't function, and it will breed ill will. And the recipient countries hold their best people back".

The approach of UN agencies, like UNEP's desert unit for example, is to encourage the individual countries to create their own projects, "so there is no question of imperialism". Furthermore the UN can call on expertise internationally, whereas bilateral agencies are limited to the donor country.

In the Sahel, however, Høgel recognises the enormous resources that the Club du Sahel can call on—some \$2,000 million compared with \$60 million in UNEP's Sahelian office—"and the beneficiaries are the same". The Sahelian population is the worst hit and also the poorest desertification area in the world.

The desert unit at UNEP is still very small. UNCOD recommended a professional staff of ten, and some think it should be larger, but so far Høgel has



Høgel: supporter of national projects

himself and five others. Previously Høgel was a development manager with UNDP in Upper Volta; he is a 46-year-old Dane with wide experience in the third world. To assist him he has a Soviet professor in soil science, a Sudanese ex-vice-minister of agriculture (and a doctor of veterinary medicine), an Afghani programme officer, and a Belgian meteorologist. The latest recruit is a Malian ex-nomad turned sociologist of nomadic populations.

The unit's work covers the six transnational projects, establishing new national projects, organising national courses on the most basic desert management, and map-making. The original UNCOD map has been widely criticised as impractical, on too small a scale, and lacking clear definitions of the desertification process. A new one at 50 to 100 times the scale (1:1 million) is being drawn up by Professor Harold Dregne at Texas Technical University, but it will take two years and is already leading to a lot of disagreement on defining borders. "But the footwork needed to arrive at the map is very important" says Høgel. "At least we ought to know in detail where the danger regions are."

Robert Walgate



"He drinks one, then needs three more to forget about the cancer in the first."

German scientists detect carcinogens in beer

SCIENTISTS at the German Cancer Research Centre in Heidelberg have discovered traces of nitrosamines, one of the strongest known cancer inducing agents, in beers made in Germany, Britain and other European countries.

A two-year study of 158 beers financed by the German government, with support from the US National Cancer Institute, revealed traces of nitrosamines in 70% of the samples. The chemical, which is thought to be

produced by contact with nitrous oxide when the beer's malt is dried, was found in much higher levels than in foodstuffs such as pickled meats, ham and sausage also included in the study.

Dark beers and stronger beers contained higher levels of nitrosamines than light beers such as lager and pilsner, according to the head of the research team, Professor Rudolf Preussman.

David Dickson

news in brief

British Association meets on UNCSTD: As preparations mount for the UN Conference on Science and Technology for Development (UNCSTD) in August, the British Association for the Advancement of Science is organising a one-day public symposium on March 8 to allow 'non-government bodies' a chance to express their opinions. Seven main speakers will put points of view ranging from that of the UN, from Guy B. Gresford, who is Deputy Secretary General UNCSTD, New York, to the 'trade union view' by Barry Sherman, Director of Research at ASTMS. Sir Ieuan Maddock, who will also chair the meeting, presents 'a British scientist's view', while the multinational companies will be covered by Mrs Doreen Wedderburn of Unilever and chairman of a CBI working group. Professor A. H. Bunting (Reading University) and Adrian Moyes of Oxfam are scheduled to present the reaction of the late-developing countries and the 'very poor' to science and technology; while Professor Robert A. Shaw, who holds the chemistry chair at Birkbeck College, London, will speak on the educational consequences of UNCSTD, in a talk that will undoubtedly range well beyond its title.

The BAAS seems confident that the meeting will be both lively and useful. With this line-up of speakers and the quality of contributions expected from the floor, a forum of this kind should be the best way of putting across the 'unbiased opinions'—in a BAAS phrase—of British scientists to the British UNCSTD delegation. (Further details from the symposium secretary, 01-734 6010 extension 377.)



Accident delays European Space Launcher: Development flights of the European Space Launcher, Ariane, have been delayed five months due to failure of a safety device on 28 November 1978. An ignition delay in the combustion chamber caused an excess of hydrogen to build up which resulted in a "violent ignition", according to ESA. The explosion caused extensive damage to the propulsion system requiring the launcher to be dismantled and refurbished. But initial fears that the stage or propulsion design might have been responsible were unfounded. An inquiry conducted by the project team found that a malfunction of a hydrogen flow monitor was responsible. The new dates for the first four development flights are: early November, 1979; early March, 1980; June, 1980; and October, 1980 (unchanged). The revised schedule permits the first operational flight to go ahead as scheduled in December 1980.

US scientists urge further intensive study of Mars and Venus: Mars, Venus and the Earth should receive the major focus in exploration of the inner solar system for the next decade, according to a report published recently by the National Research Council, the study section of the US National Academy of Sciences. "The comparative planetology of these bodies is a key to understanding of the formation of the earth, its atmosphere and oceans, and

the physical and chemical conditions that lead to the origin and evolution of life", the report says, although it adds that the atmosphere-free terrestrial planets, namely Mercury and the Moon, are "complementary bodies of high scientific interest".

As for further missions to Mars and Venus following the Viking missions of 1977 and the current Pioneer missions, the report says that studies should be initiated to develop the special technology needed for the return of samples, as well as *in situ* experiments. In an "overview" section of the report, the committee says it is deeply disturbed by the near-term outlook for the US planetary programme, and that the struggle in 1978 to obtain approval of the Jupiter Orbiter Probe, after a three-year hiatus in planetary mission starts, was a reminder that a national policy for planetary exploration was still to be defined.

The report also says that in view of the lack of direct and substantial information on Soviet plans for planetary exploration, and because of the limited extent of past scientific exchanges, "it appears desirable to establish a much improved vehicle for discussion of US-USSR interests in planetary exploration".

US to assess environmental impact of foreign projects: All US federal agencies must in future prepare an assessment of the likely environmental impact of any new project to be carried out in a foreign country, following an executive order signed last week by President Carter in Washington. Projects that could be affected by the order include the construction of a nuclear reactor in the Philippines, which critics claim to be dangerously near an earthquake fault, and US participation in spraying Mexican marijuana fields with the herbicide paraquat.

The presidential order follows almost a decade of dispute over whether the National Environmental Protection Act of 1969, which requires environmental impact statements on federal projects within the US, was also intended to cover projects carried out overseas. The Council on Environmental Quality has always maintained that this was, indeed, the intention of the US Congress in passing the Act; in contrast, the US State Department has claimed that carrying out such an assessment would be a violation of the sovereign rights of other countries. Dr Charles Warren, chairman of the Council on Environmental Policy said that the order was a response to a growing worldwide concern that governments are undertaking major actions without enough consideration of the consequences.

Libyans deny A-bomb intentions: Libyan officials have denied charges by the US Federation of American Scientists that the country intends to acquire nuclear weapons. According to Mr Ahmed Shehata, head of the foreign affairs office of Libya's ruling People's Congress, Libya has "neither the time nor the potential" to acquire nuclear weapons itself, although it believes that the superpowers should curb any further transfers of nuclear-weapons technology to Israel, South Africa or Rhodesia. The charges were made after a trip to Libya two months ago by FAS secretary Dr Jeremy Stone.

Birmingham University challenges smallpox trial: Birmingham University has won leave to apply to the High Court to quash its prosecution over the smallpox case. The university claims the trial has been prejudiced by the premature release of the Shooter report on the incident.

No crisis, but save energy, say Soviets

Vera Rich reports on Soviet energy in a year when nuclear generating capacity in the USSR is to increase by 50%.

DURING the past year, Soviet energy planners have issued a number of highlevel denials of anything approaching an energy crisis. Since the publication of the 1977 report of the US Central Intelligence Agency which predicted that the Soviet Union would become a net importer of energy by the late 1980s, considerable effort has been put into official denials.

And yet in 1976 a major publicity campaign was launched to convince the population of the necessity to save energy in all its forms. According to official policy, this does not imply an energy crisis—it is simply intended to make people aware of the need to use the natural resources of the country “rationally”. The policy-makers insist that sufficient energy resources exist, and any temporary set-backs are due to the geographical and logistical problems of exploiting them or, alternatively, slackness in some vital part of the energy industry or its auxiliaries.

Such slackness, even at the highest levels, is not to be tolerated. At the end of December, the Minister of the Coal Industry of the Ukrainian USSR was dismissed on the grounds that his ministry had been unable to rectify the production lag in the coal industry. For some years coal occupied a less elevated place in Soviet planning than other forms of energy, but it now appears to have been re-emphasised. Prospecting for new coal deposits has been going on, not only in the unexplored regions of Siberia, but also in the European parts of the USSR. During 1978, two major new coalfields were discovered at Lisichansk and Bogdanovskoe in Ukraine, conveniently close to traditional centres of industry. It is hoped both will be fully productive by 1985.

Unfortunately, most major energy sources in the Soviet Union are not so conveniently placed. The main centres of population lie west of the Urals; the new oil and gas fields lie far to the east. There are clear indications that the exploitation of these deposits is proving far more costly, both in money and in time, than was originally expected.

In January 1978, a group of foreign journalists on an official tour of the west Siberian oil fields, ‘accidentally’ met Sergei Velikopol’skii, First Secretary of the Communist Party branch at the administrative headquarters of the Samotlor field, the largest oil field in the Soviet Union. It is true, he admitted, that the output

of the Samotlor field will level off over the next seven or eight years, and will then decline, but this will be more than offset by bringing the east Siberian fields on-stream. The fact that production from Samotlor will peak earlier than expected is merely due to the extreme climatic conditions encountered in the east Siberian fields. In order to meet production plans, more oil has had to be taken from Samotlor than originally projected for the current planning period.

In spite of the apparently optimistic tone of this interview, Velikopol’skii’s remarks seem to suggest delays in the exploitation of the east Siberian fields. Permafrost or swampy terrain hardly provide optimum drilling conditions—although in the latter case, there has been encouraging progress in the use of slant drilling from artificial islands. Transporting the oil westwards is, however, an even more costly business. According to Academician Mikhail Styrikovich, it costs about 1 million roubles (a rouble is about \$1) to lay 1 km of pipeline in this terrain, while the construction of workers’ settlements costs 50–100% more than the construction of similar settlements in developed areas.

Sometimes, indeed, the logistics of oil extraction become ludicrous. At the Urengoi gas deposit, not only did the base camp have no proper maintenance facilities for equipment, all fuel for motor vehicles and tractors had to be hauled some 2000–3000 km, so that the transport costs exceeded those of the fuel itself. Only after some considerable time was it realised that motor fuel could be produced on site from the gas itself, and an experimental unit, capable of producing some 35 tonnes of fuel every 24 hours was installed on site.

Although unwilling to admit to an energy crisis, the Soviet authorities do admit, under pressure, to having underestimated their production costs. This January Moscow radio, replying to an article in *Le Monde* which censured recent increases in the price of Soviet oil to the Comecon block, admitted an additional investment cost of 7,500 million roubles, simply to keep up the current level of supplies to Comecon. And this, it claimed, was in spite of there being no transport cost, since the outlay on the “Friendship” pipeline had already been recovered.

Furthermore, comparison of the official figure for pipeline built during the five-year-plan 1971–75 (12,000 km) and the amount of large-diameter pipe

bought from abroad (1,000 million tons—sufficient to build some 13–15,000 km of pipeline, depending on wall-thickness), it would appear that the pipelines were at that time built largely from imported pipes. (The situation may have changed recently, judging from complaints last year that certain Soviet factories charged with producing the required pipes had failed to do so, thus delaying pipe-laying operations). Add to this auxiliary technology purchased abroad—high capacity compressor stations (Rolls-Royce), pumps for water-flooding techniques (US), desulphurisation equipment (France)—and the expenditure of precious foreign currency reserves must be considerable.

Even the Soviet Union’s “prestige” position as major oil supplier to Comecon, has been falling since the sudden increase of oil prices to the bloc, in September 1976 (following pledges not to increase). While still paying lip-service to an integrated oil and gas policy for the Comecon bloc, a number of the individual countries are quietly pursuing their own alternative plans—East Germany the underground gasification of coal, Poland a country-wide survey for oil and gas resources, and Romania an off-shore drilling programme.

However, if the great pipelines, with their evocative names of “Friendship”, “Brotherhood” and “Union” are more expensive as a means of integration than was first hoped, there still remains the new electricity link between Vinnitsya in Ukraine and the Comecon terminal at Albertirsa in Hungary. The Soviet Union has considerable plans for expanding generating capacity.

A unified power grid for the country is being completed, which would obviate any major differences in peak and non-peak hours. Since the Soviet Union is so wide east to west, there is virtually always a peak period going on somewhere in the country, to which electricity could be relayed from some less busy region. (The possibility of cryogenic superconductor transmission is seriously under consideration.)

Speaking on “Power Workers’ Day” last December, Petr S. Neporozhnyi, the Minister of Power and Electrification, told *Pravda*, that in 1979, the generation of electric power is to be brought up to 1,265 GW, which would include an almost 50% increase in nuclear power generation.

The stress on the nuclear branch is not accidental. In spite of the unified grid, it would clearly be more convenient to generate electricity close to where it is required. The major sources of fossil fuel for thermal power



Drilling in Siberia (left) is tough enough: then comes the problem of getting the gas and oil back to population centres.

stations, and the large rivers still not committed to their full hydroelectricity capacity lie far to the east—but a nuclear power station in the Soviet planners' opinion, can be built wherever required.

For the Soviet planners do not have to contend with an antinuclear lobby; even the dissidents seem to have accepted that nuclear power is an intrinsic good (unlike the Czech Chartists who recently demanded a referendum on the whole issue).

Repeatedly, official spokesmen stress that nuclear power stations are safe and do no damage to the environment. A book published in 1974 to celebrate 20 years of nuclear power generation did, indeed, contain a chapter on safety, but this only refers to radiation protection within the plant itself.

The Soviet planners seem convinced that no major accident can possibly occur. According to one US nuclear delegation, which visited the Soviet Union in 1970 "Reactor design is based on the assumption . . . that there is no credible accident corresponding to a severe accident excursions or total loss of coolant . . . The safety philosophy for light water reactors is similar to that for the LMFBR's in the USSR. For example, a double ended pipe break is considered incredible; therefore, no separate emergency core cooling systems, no containment buildings, no ice condensers, and no pressure suppresant chambers are provided".

With this somewhat casual attitude to safety, the Soviet Union is embarking on a major nuclear power programme. Last year new power sets were commissioned at the Leningrad, Novovoronezh, Chernobyl and Kursk power stations, and at present 13 nuclear power stations are under construction. Set capacity is to be

increased from 440 MW to 1000 MW and there is even talk of sets of up to 2500 MW being feasible.

Most of the new stations are RBMK uranium-graphite boiling water reactors or water-cooled, water-moderated reactors. However, the present five-year plan commits the economy to the development and construction of fast-breeder reactors.

A pilot 12 MW fast breeder was constructed in Leningrad in 1968, and at Shevchenko, on the Caspian sea, a fast-breeder has been in operation since 1973, acting as the sole source of electricity and water desalination for the town. This was the reactor which in 1974 was observed by US surveillance satellites to have been damaged by an explosion although, according to official Soviet sources, their satellites failed to record anything amiss and the local population "did not even know that anything had happened". (Unofficial sources, however, reported considerable panic). Even when an off-the-record comment on the accident can be obtained, it is always presented as a small conventional explosion due to the contact of liquid sodium and steam in the heat exchanger—not properly speaking a nuclear event at all.

A second FBR being built at Belyarsk was originally scheduled to be completed in 1973, but will probably be commissioned this year. Both these FBRs are uranium-based, but according to Mikhail Troyanov, Deputy Director of the Institute of Physics and Power Engineering "we see no difficulty in moving to plutonium. After 1990, breeders should be built in the USSR in large numbers".

Not only are the Soviet planners prepared to site power stations in centres of population; they are equally ready to use nuclear power to provide

district heating, and as an industrial steam source. One such system is already operating at Bilibino on the Chukotka peninsular. Most commentators assume that such installations would be a combined source of electricity and heat, but mention has also been made of a special low-temperature, low-pressure water cooled reactor which could deliver 500 MW heat but no electricity, and which could possibly go into production at the beginning of the 1980s.

Against this background of expansion, there is a constant drive for more and more fuel economy. A number of less conventional energy sources are under investigation—windpowered generators are already being manufactured for use in the sheep-rearing areas of Moldavia and the far north, and the possibility of constructing solar power stations in the arid steppe is receiving serious attention. At the Institute of High-Temperature Physics, research is going forward (with US cooperation) on MHD generation as a means of utilising fossil fuels more efficiently.

The Far Eastern Science Centre is working on the possibility of using geothermal heat—in accordance with high level directives that they should devote their main attention to the problems and potentialities of their own area.

In spite of the expected advent of the supergrid, the possibility of pumped storage stations has not been overlooked, and the construction of such a station at Zagorsk was begun last September. And, since the fuel-saving campaign was inaugurated in 1976, (it was intensified in September 1978) there has been a constant discussion in the media of possible means of economising. These range from the domestic—one appeal on television to turn off unnecessary appliances is said to have saved 10 MW in a single hour—to new means of using fuel. The latter range from genuine savings—such as the saving of "more than 17%" of all thermal energy needs at the Kemerovo fertiliser works by using the heat from exothermic reactions, to advice about burning maizestalks or woodchippings, which seem more a symbolic gesture than a piece of practical economy.

Nor is the energy industry prepared to shoulder all the burden. One letter to *Pravda* from a manager in the Leningrad power industry suggested that it is not the power workers who should think of energy-saving, but the architects and designers. Their "window-mania" of the last few years, he claimed, has caused unnecessary increases of up to 60% in the cost of heating living and working space! □

correspondence

Short term contracts

SIR,—It is sad that three recent articles (23 November, page 310; 28 December, pages 743 and 745) on the difficulties facing research workers in universities have induced little dialogue in your correspondence columns. It has been our association's experience that there are at least two reasons for such a lack of response: (a) research scientists are often isolated in departments with a high percentage of tenured staff and think that protestation would have little or no effect; (b) many fear that if they do step out of line and "speak out" they will not only jeopardise their present job but will find it even harder to secure another. Presumably, it is because of this that the present appalling situation has been allowed to develop. We therefore would like to appeal to all researchers to start thinking positively, join a union and become active in it, join our association (if appropriate), or form an association and write to your MP, asking for an inquiry. We demand to have a career structure in research with some prospect of security so that a PhD will come to be regarded again as an attribute and not a liability!

ARLEEN UNGER

ARMS, London, UK.

Sollas and the Sherborne bone

SIR,—I would like to correct the record of the Sherborne engraved bone controversy, unfortunately misrepresented in Dr L. B. Halstead's account of the late Professor Douglas's tape-recording linking Sollas with the Piltown hoax (2 November, page 11). Douglas's story reveals nothing fresh except his own assumption that Sollas kept quiet about a 'hoax' at Sherborne School (Dorset) so that Smith Woodward might make a fool of himself; the rest is a tendentiously garbled summary of Bayzand's evidence against the bone in *Nature* 117, 233; 1926, confounded with facts stated by Smith Woodward himself (*Nature*, 117, 86; 1926).

Dr K. P. Oakley established in 1957 that the piece of bone was ancient and consistent with an Upper Pleistocene date. It is clear to the naked eye that it is semi-fossilised, as Smith Woodward claimed in his reply (*Nature* 117, 86) to Sollas's denunciation in *Ancient Hunters* (1924). Sollas (*Nature* 117, 233) was nonplussed by this claim and attempted to parry it with an ignorant remark betraying that he had never seen the object. Neither had Bayzand! Had he done so he could not have left Sollas in ignorance of a fact so vital to any assessment of a schoolboy hoax, or failed to investigate an ostensible fake of extraordinary ingenuity, as his wholly imprecise charge shows. It is impossible to resist the conclusion that Bayzand heard a story, true or false, and simply did nothing at all—until 1914, as he himself tells us, when

Smith Woodward's paper appeared (*Q. Jl. Geol. Soc.*, 70, 100–2).

It is generally supposed that Sollas and Bayzand were left with the last word, for Douglas evidently believed them, as did the late Joseph Fowler of Sherborne, whose exhaustive but uncompleted research led to my own interest in the affair. There was, however, a third letter in *Nature* (117, 341–2; 1926) only weeks later, from the supposed hoax victim, R. E. Steel, who began by apologising for Bayzand's having been misled by senior boys who had been unable to credit such a find by two 14-year-olds, Arnaldo Cortesi and Philip Grove, in their first weeks at the school. His account of the circumstances of discovery, corroborated by a former pupil who had recognised the character of the engraving and prevented Cortesi from throwing it into the day-room fire, and the aptness of the alleged site (thereupon efficiently investigated by Steel), are entitled to respect.

The question remains whether there was anything more than school rumour in the story Bayzand treated so casually but with such disastrous consequences for a potentially rare and interesting find. Was the engraving nevertheless faked, and if so, whence came the material and expertise? One can imagine Douglas's answer—one that would at least make sense of his otherwise forced analogy between Sherborne and Piltown—but I hope shortly to deal more fully, in *Antiquity*, with this and other aspects of this curious tale.

R. A. H. FARRAR

Royal Commission on Historical Monuments,
London, UK.

Seveso

SIR,—Professor Garattini and Dr Manara of the Istituto di Ricerche Farmacologiche Mario Negri, Milan, have accused me of underestimating the possible damage to human health caused by the Seveso accident (7 December, page 556).

I took on the assessment of the damage to health caused by the accident because I had been personally involved from the beginning in protection and prevention measures for the injured and for those still exposed.

I played an important role in the decision to evacuate the population of Seveso. This has been stated in several books on Seveso and in official documents in my possession. I never expected that readers of my papers would accept on sight my assessment of the consequences of the accident on the health of the population, and I have therefore always based my reports and conclusions on documents^{1–10} which cannot be accused of prejudice in my favour. The great majority of these authors are either members of the Epidemiological Medical Commission of the Lombardy Region in Milan (of which Professor Garattini has been a

member since its beginning) with responsibility for the health surveillance of the population, or of the Advisory Scientific Committee to the Italian Government in Rome for the Seveso accident (Commissione Cimino).

They have been (and still are) confronted with one of the most difficult and tricky problems in medicine. They have mastered the emergency with courage, skill and competence. The dermatological screenings carried out by them on 32,000 schoolchildren have no precedent in the history of clinical toxicology. I trust their reports to be accurate, meticulous and unbiased.

Nevertheless I may have overlooked or missed data available to Professor Garattini and Dr Manara which would prove me wrong. I therefore ask Professor Garattini and Dr Manara to make any such data known immediately, because only then (and not by saying that I am biased) will it be possible to evaluate the damage.

About 5,000 people are still exposed to levels of TCDD which, while admittedly lower than those of past cases of occupational exposure, could represent a potential risk. If my conclusions are wrong, we have a moral obligation to take protective measures immediately to prevent any further damage to this population.

G. REGGIANI

Hoffmann-La Roche

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news and views

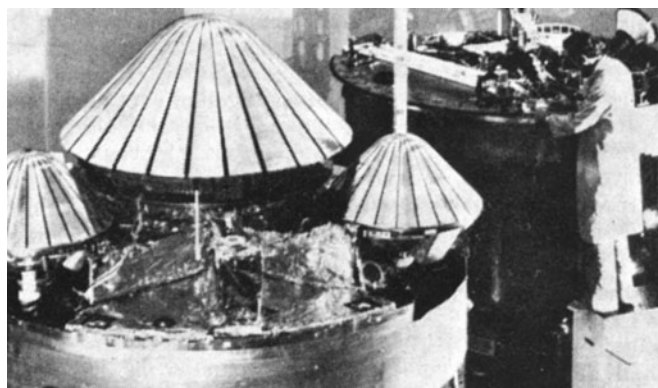
Epithelial cancer, differentiation, and vitamin A

from Brigid Hogan

MORE than 90% of people who die of cancer are killed by metastasising tumours of epithelial tissues. These malignancies do not develop rapidly, but evolve over one or two decades through stages in which there is increasing disorganisation of the epithelial layer and its association with the underlying stroma. Studying this latent period is like piecing together a complex and tantalising jigsaw puzzle; complex, because the pieces represent all the agents such as hormones, growth factors and tumour promoters which affect the proliferation, organisation and differentiation of epithelial cells; tantalising, because we obviously still lack many of the vital pieces needed to complete the picture and so perhaps learn how to intervene therapeutically between the initiating event and the final malignant state. One piece of the puzzle are the retinoids, a class of compounds based on vitamin A which for a long time has been known to have profound effects on many epithelial tissues.

Retinoids in organ cultures

The effect of retinoids on epithelial cells has been studied for many years. In young animals vitamin A deficiency results in the transformation of simple, ciliated, mucous-secreting epithelia (such as those found in the respiratory and genital tracts) into multilayered, non-secretory keratinised epithelia, a process known as squamous metaplasia. The change can be prevented (or reversed) by adding retinoids at concentrations down to about 10^{-10} M (see Clamon *et al.* *Nature* **250**, 64; 1974, for the standard assay using pieces of trachea from vitamin A-deficient hamsters). With higher concentrations of retinoids the tracheal cartilage starts to break down and proteases and hydrolases released from the chondrocytes begin to digest away the matrix. This response is usually con-



sidered to be simply the result of the detergent-like action of high concentrations of polar retinoids on the cell membrane, but others might argue that it reflects a physiological response of chondrocytes, important in tissue remodelling during early development. Of the natural retinoids, retinoic acid is the most effective in reversing squamous metaplasia and can be given in much higher doses as it is not stored in the liver.

Almost nothing is known about the way in which retinoids mediate their effect on epithelia. For example, they could act on the epithelial cells directly, or indirectly by provoking signals from the underlying stromal cells. It is possible that, like the steroids, the retinoids have to enter the cell to be effective; several cytoplasmic retinoid-binding proteins have been isolated from a variety of whole organs, and the affinity of one of these proteins for different analogues correlates with their effectiveness in preventing squamous metaplasia in organ cultures (Chytil & Ong *Nature* **260**, 49–51; 1976).

Retinoids and carcinogenesis *in vivo*

One of the most exciting new ideas in the study of retinoids is the possibility that they can intervene in the process of epithelial carcinogenesis *in vivo*. Several months after giving a dose of carcinogen to experimental animals, the target epithelium shows areas of disorganisation, ranging from hyperplasia (increased mitotic rate of the basal cells), squamous metaplasia and papillomas, to increasingly invasive carcinomas. A number of groups have

reported that feeding the animals high doses of retinoids after the carcinogen treatment reduces the severity and often the incidence of the lesions in a variety of epithelial sites (for example, bladder, lung and trachea) (for reference, see Sporn *et al.* *Fed. Proc.* **35**, 1332; 1976). In these experimental systems, retinoic acid has proved to be more effective than other natural vitamin A derivatives. (Some workers have reported that retinoids have no effect on tumour incidence, but Sporn has argued that this is because retinol derivatives that can be removed from the circulation and stored in the liver were used). The action of retinoids therefore seems to be exactly opposite to the effect of tumour promoters—naturally occurring chemicals like the phorbol esters which do not themselves cause cancer but increase the rate at which tumours appear in response to carcinogen treatment (see *News and Views* **270**, 659; 1977). The retinoids, in other words, seem to be acting as 'anti-promoters'. But before readers run off to order mega-doses of retinoic acid, it must be stressed that the story is by no means simple, and there are reports that retinoids can enhance tumour growth, although in these cases the vitamin derivative was applied in high and possibly irritant doses to the skin rather than included in the diet (Polliack & Levij *Cancer Res.* **29**, 327; 1969; Polliack & Sasson *J. natn. Cancer Inst.* **48**, 407; 1972).

Retinoids and *in vitro* cell cultures

The problem with studying the action of retinoids *in vivo* or in organ culture is that there are too many pieces to the

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puzzle. One way round this is to look at the effect of retinoids, either alone or in combination with purified growth factors and tumour promoters, on cell lines in culture, when there is only one target cell type. For example, retinoids can be shown to restore anchorage dependent growth to some transformed cells (Dion *et al. Expl Cell Res.* **117**, 15; 1978) and inhibit the growth of melanoma cells (Lotan *et al. J. natn. Cancer Inst.* **60**, 1035; 1978).

In a recent paper, Todaro *et al.* (*Nature* **276**, 272; 1978) reported that retinoids inhibit the mitogenic effect of sarcoma growth factor (SGF) on a line of untransformed rat fibroblasts. Earlier studies had shown that SGF production is switched on when cells are transformed by murine sarcoma virus. The polypeptide seems to promote cell division (either in the same cells or in other, normal lines) by binding at the plasma membrane to the site normally awaiting the arrival of epidermal growth factor (in spite of its name, EGF promotes cell division in a variety of 'fibroblast' cell lines as well as in epithelial cells). It has been argued that tumour promoters mimic SGF and EGF by short circuiting the membrane-intracellular effector signal sys-

tem (Lee & Weinstein *Science* **202**, 313; 1978), and the results of Todaro *et al.* suggest that the retinoids may block the circuit altogether. Although still very speculative this model accounts for the opposite effects of retinoids and tumour promoters *in vivo*.

As usual though, things are not as simple as they seem and far from antagonising the effect of various promoters, it seems that retinoids can act like promoters in certain systems. For example, Wilson and Reich have recently described a culture system in which retinoids act synergistically with phorbol ester and Rous sarcoma virus in stimulating plasminogen activator in chick embryo fibroblasts (*Cell* **15**, 385; 1978). Plasminogen activator is an extracellular protease which is assayed *in vitro* by its ability to digest a fibrin clot, but very little is known about its role in fibroblast behaviour in the whole animal. It seems very likely, for example, that the enzyme is important in tissue remodelling during growth and wound healing. Until more is known about the effect of retinoids on these processes *in vivo*, Wilson and Reich argue for caution in interpreting all results according to a simple model.

Indeed, the same issue of *Cell* adds

another facet to the problem of retinoid action in the form of a paper by Strickland and Mahdavi on the effect of retinoids on the differentiation of murine teratocarcinoma cells *in vitro* (*Cell* **15**, 393; 1978). These authors claim that retinoic acid promotes the differentiation of F9 embryonal carcinoma cells into parietal endoderm cells, which normally synthesise plasminogen activator and large amounts of basement membrane material. Similar results have been observed in M. Sherman's laboratory using a variety of synthetic analogues of retinoic acid (Jetten & Jetten *Nature*, in the press). Although the results suggest that retinoids can increase the (normally low) probability with which F9 cells switch to endoderm, the possibility that they are increasing the survival of endoderm cells formed 'spontaneously' in the cultures has not yet been completely eliminated.

Meanwhile, readers who find these multifarious results too indigestible might ponder on a paper by Graham *et al.* (*J. natn. Cancer Inst.* **61**, 709; 1978) who report a lower risk of cancer of the colon and rectum associated with high intake of green vegetables. □

Free-electron lasers

from J. D. Lawson

SOURCES of incoherent radiation have been familiar since scientific enquiry began; the generation of coherent radiation, on the other hand, is a relatively recent achievement. Fifty years ago, coherent radiation with frequency up to about 10^7 Hz was available, and in common use for radio transmissions. More recently, however, a series of inventions, culminating in the laser, have pushed the frequency up by many orders of magnitude to the ultraviolet region of the spectrum.

Devices invented during the past 40 years include the klystron, cavity magnetron, travelling wave tube, ubitron, ammonia maser and, at higher frequencies, the optical laser and free-electron laser. All depend on the stimulated emission of photons. If the photons interacting with the charges are real, they constitute a radiation field, and the device may be termed a 'laser'. In some systems the electrons are essentially free, or move in orbits determined by external magnetic fields; in others they are bound to atoms in gases or in

solids. Some devices require a quantum mechanical description, whereas others do not. The first analysis of the free electron free-electron laser was in quantum mechanical terms, but classical theory is sufficiently accurate and is now always used.

The operation of two systems described as free-electron lasers has been reported, one at Stanford University (Deacon *et al.*, *Phys. Rev. Lett.* **38**, 892; 1977) and the other at the Naval Research Laboratory, Washington (McDermott *et al.*, *Phys. Rev. Lett.* **41**, 1368; 1978). These devices operate in different regimes, and both form an interesting link between conventional lasers and microwave tubes.

In most microwave generators the beam interacts with single mode cavities, or with structures carrying a slow wave with longitudinal phase velocity close to that of the electrons. Such cavities and structures are limited to transverse dimensions of less than about a wavelength, and this imposes a lower wavelength limit of a few millimetres for reliable devices with useful power output. This limit may be avoided by making use of interaction

with a 'fast' electromagnetic wave, which has phase velocity equal to or exceeding that of light. Such interaction necessarily implies a velocity difference between wave and particles; although not synchronous, coherent interaction can nevertheless be obtained by modulating the orbit of the electrons. This principle (Gorn *U.S. Patent* 2, 591, 350, filed 1947) has been used in the 'ubitron' millimetre wave generator, (Phillips, *ITED*, ED-7, 231; 1960), and the free electron laser represents a special application for relativistic electrons when the fast wave is essentially a plane wave travelling along the axis. I shall now describe the Stanford scheme.

Suppose that a circularly polarised light wave of wavelength λ travels in the z -direction. A particle travelling along the z -axis experiences a transverse force which rotates with angular velocity proportional to the difference between the electron velocity and the light velocity. If now a twisted transverse magnetic field of periodicity D is introduced on the axis (with field vectors corresponding to the edge of the stairs in a spiral staircase) the

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particle is constrained to move in a spiral orbit. If this spiralling is synchronous with that of the electric field vector as 'seen' by the particle, then coherent interaction and continuous energy transfer is possible. For highly relativistic particles moving in a helix of radius $r \ll D$, simple geometrical arguments show that

$$\frac{\lambda}{D} \approx \frac{1}{2\gamma^2} \left(1 + \frac{4\pi^2 \gamma^2 r^2}{D^2} \right)$$

where $\gamma m_0 c^2$ is the electron energy and $\gamma \gg 1$. Thus, for reasonable values of D , (a few centimetres), and γ of order 50–100, coherent interactions may be obtained in the infrared or optical region. Freedom from the limitations of slow-wave systems has, however, been bought at the expense of a relatively weak interaction. This is because the electric field is almost perpendicular to the particle orbit.

A beam of electrons moving at approximately the synchronous velocity exchanges energy with the wave; if the average particle velocity is slightly greater than the synchronous velocity, energy transfer from the particles to the wave is possible. Two regimes may be distinguished, the low current regime in which the wave interacts essentially with individual particles, (as in an accelerator) and a high-current high-gain regime where the plasma oscillation frequency in the beam is no longer negligible, and the interaction is with a space-charge wave travelling on the beam. The Stanford and Columbia-NRL lasers referred to earlier operate respectively in these two regimes, which can be described in quantum language in terms of Compton and Raman scattering.

The first demonstration of the interaction mechanism outlined above was by Madey and his team at Stanford in 1975 (*Phys. Rev. Lett.* **36**, 717; 1976). Making use of the superconducting linac there, which is able to provide a continuous succession of short high quality pulses of electrons, amplification of 10.6 μm radiation from a CO_2 laser was demonstrated. The twisted magnetic field of 0.24 Tesla was provided by a superconducting bi-filar helix, of 5 m total length. This experiment was followed in 1977 by a further demonstration, using the same magnet and accelerator, of a laser oscillator. Mirrors 12.7 m apart were placed symmetrically about the helix, and the electrons were steered by magnets to avoid them at either end. A mean power of 0.36 W was measured, corresponding to a peak power of 7 kW during the pulse. The wavelength was 3.14 μm , and the linewidth of 0.23% was determined essentially by the very short pulselength of 4 picoseconds.

Such a laser has the very attractive feature that it can be tuned by varying the electron energy. Unfortunately, however, the apparatus required is expensive, and the overall efficiency is low. Furthermore, the linewidth is rather broad for spectroscopic applications. The use of a storage ring to recirculate the beam and thus raise the efficiency has been suggested. Unfortunately, passage through the laser induces energy spread in the beam and this greatly reduces the efficiency on subsequent transits. Whether, and if so how, this effect can be overcome is still a subject of active debate.

It is too early to foresee what the applications of this type of laser will ultimately be. More study, both experimental and theoretical, is required before realistic assessments of power and efficiency limitations can be made; the gain decreases rapidly as γ increases and it remains to be seen just how the various technical constraints conspire to produce a lower wavelength limit. Experiments are difficult, and progress may not be rapid.

The laser recently described by groups at Columbia University and the Naval Research Laboratory, operates in the collective regime. A rippled solenoidal magnetic field is used, and interaction is with the slow, negative

energy space-charge wave on the electron beam. (The system may be viewed in a frame of reference moving with the electron as a parametric amplifier, in which the rippled field and space-charge waves play the part of pump and idler.) The current in this laser is 25 kA, (about 10^4 times that in the Stanford laser), but the energy is much lower, corresponding to $\gamma=3.2$. 1 MW pulses of radiation of length 20 ns and wavelength 400 μm were extracted through the hole in one of the mirrors. This is not the first observation of microwave power from intense relativistic beams in a rippled field; it is however the first time that a laser cavity has been used to provide feedback and enhance the output. The spectral bandwidth was 2%; this arises from the difficulty of maintaining constant beam conditions during the pulse.

Whatever the final applications of the radiation from these devices, (a topic not discussed in the papers quoted), they form a promising addition to the available range of submillimetre generators. The latest volume of 'Physics of Quantum Electronics' (5, Addison-Wesley, Reading, Massachusetts, 1978) devoted entirely to this field includes an interesting variety of points of view of the theory, and a historical summary. $\square$

Contractility of the roots of flagella and cilia

from Michael Sleight

A HIGH proportion of the basal bodies of cilia and flagella bear striated roots and/or bundles of microtubular fibrils. In the absence of clear evidence for any other function it has been assumed that the purpose of these attached fibrous structures is to provide a firm anchorage for the ciliary base, so that the propulsive activity of the ciliary shaft can be exploited to the full. However, large striated roots are present not only on mechanically active cilia and flagella, but also on such sensory cilia as those in the rod cells of the vertebrate retina, where anchorage of the ciliary base would not seem to require such a large root structure. The recent report by Salisbury and Floyd (*Science* **202**, 975; 1978) that the striated roots of flagella of the algal flagellate *Platymonas* undergo a calcium-induced contraction should provoke further investigation of these structures.

The variation in the spacing of the major bands of striated roots has puzzled some electron microscopists for

about 20 years; not only is there a wide range of periodicities in different species, varying between about 12 nm and about 200 nm, but there are also variations within species of between 30 and 60 nm in *Trichonympha* (Pitelka & Schooley *J. Morph.* **102**, 199; 1958) and between 13 and 25 nm in *Naegleria* (Simpson & Dingle *J. Cell Biol.* **51**, 323; 1971). Many authors noted a superficial similarity to collagen in the periodicity and appearance of these fibres, since in many cases periodicities of between 60 and 70 nm with several intraperiod bands were found, but the detailed banding pattern is different and a biochemical analysis of roots from mollusc cilia by Stephens (*J. Cell Biol.* **64**, 408; 1975), revealed the presence of a novel type of protein (ankyrin) that could be separated as a doublet with molecular weights of 230,000 and 250,000. Each striated root is composed of a bundle of longitudinal filaments about 5 nm thick, sharing the common banding pattern.

The two striated roots of *Platymonas* pass from the basal body complex at the base of an anterior depression of

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the cell across the centre of the cell close to the nucleus and terminate near the plasmalemma at the posterior end of the cell. The periodicity of these roots ranged from an extended spacing of 180 nm to a contracted spacing of about 60 nm. The longitudinal filaments were not visible in contracted roots, which were substantially (30%) fatter than extended roots. The extended pattern was seen in cells fixed after washing in artificial seawater lacking calcium or after washing in solutions containing EGTA, while the extreme contracted pattern was seen in cells treated with artificial seawater containing 2 mM CaCl_2 ; several other divalent cations were partially effective in causing contraction. Living cells observed in artificial seawater containing both 2 mM CaCl_2 and 5 mM ATP could be seen using the light microscope to undergo cyclic dimpling of the cell surface at sites of root attachment, and fixed cells from such experiments showed intermediate periodicities. This cyclical contractile activity of the striated roots is of particular interest, but the authors do not state its frequency, nor whether it coincides with any phase of the flagellar beat cycle. It is also of interest that the external application of ions and ATP is adequate to stimulate the contractile response in intact cells. The fully extended roots may be abnormal, since seawater contains calcium ions equivalent to about 10 mM CaCl_2 .

Salisbury and Floyd suggest that the contraction of flagellar roots of *Platymonas* may have a function in the co-ordination or directional control of flagellar beating. This is interesting because in *Chlamydomonas* the two flagella are inserted at an angle, with the two basal bodies forming a V and a thick striated fibre (periodicity about 80 nm) connecting the upper parts of the two basal bodies (Ringo *J. Cell Biol.* **33**, 543; 1967). The response of *Chlamydomonas* to stimulation with calcium ions is a narrowing of the angle between the two basal bodies, perhaps by contraction of the thick striated fibre, and a transition from a 'breast-stroke' (cilium-like) beat that produces forward swimming to symmetrical undulations of the more parallel flagella that cause backward swimming (Hyams & Borisy *J. Cell Sci.* **33**, 235; 1978). It is also not impossible that the ciliary reorientation associated with the calcium-induced beat reversal of *Paramecium* could be associated with a contraction of the striated kinetodesmal rootlets (Naitoh *J. gen. Physiol.* **51**, 85; 1968).

Contractile activity of the striated roots of flagella and cilia of protists is in itself of interest, but if the striated roots of cilia in general are contractile,

this finding could have wide implications, not least for sensory physiologists. For example, in the rod cells of the human eye a large striated root is attached to the basal body of the short cilium that connects the light-sensitive outer segment to the inner segment which leads to the sensory nerve; this root penetrates deep into the inner segment and ends in close contact with the plasmalemma of the inner segment. Some years ago Matsusaka (*J. Cell Biol.* **33**, 203; 1967) showed histochemically that the ciliary roots in human rod cells possess ATPase activity. It may not therefore be too rash to speculate that the striated roots of the cilia in rod cells may be capable of contraction and that the cilium and its contractile root may be concerned in the transmission of information from the photoreceptive membranes of the outer segment to an electrically excitable membrane of the inner segment. □

Mediaeval meteors

from David W. Hughes

ASTRONOMICAL historians have been doing well during the past year, one of the high spots being the solution of a famous longstanding problem. No report had come to light in Europe and the Near East of any observations of the Crab supernova explosion which the Chinese apparently saw with great ease; it was first recorded on 4 July, 1054 and was so bright that it could be seen during the day-time for 23 days. K. Brecher, E. Lieber and A. E. Lieber solved this puzzle (*Nature* **273**, 728; 1978) by finding a reference to the supernova in a biographical encyclopaedia of physicians written in about 1242 by Ibn Abi Usaybia. The compiler told how Ibn Butlan, a Christian physician, had seen the supernova when he was staying in Constantinople and had regarded it as being the cause of an epidemic which led to the death of many thousands of people at that time.

A less newsworthy but more typical example of the work of the astronomical historian has been published recently in the *Journal for the History of Astronomy* (**9**, 123; 1979). Umberto Dall'Olmo has scoured the European annals and chronicles that have been collected in the *Monumenta Germaniae historica* and the *Rerum Italicarum scriptores* and has compiled a list of all the meteorites, meteors and meteor

showers that occurred before 1500 AD and were regarded as being of sufficient importance to be recorded for posterity.

Dall'Olmo emphasises that his list is by no means complete. First it is obvious that many events went unrecorded. Second it is a hopeless task to examine all the historical and literary texts which mention astronomical events. The discovery of the Arabic record of the Crab supernova nearly a millenium after its occurrence is ample evidence of that.

Considering these facts it is fair to ask if lists of ancient astronomical events are of any use to the present astronomical community. The answer is an unequivocal yes. The period of accurate photographic and telescopic observations is so short in comparison with the age of civilisation, never mind the age of the Earth, that anything that extends this period is worthwhile. For this reason alone people have been searching for records of solar and lunar eclipses, comets, aurora, sunspots and meteoric phenomena. In the specific field covered by Dall'Olmo the occurrence, strength and date of meteor showers are vital parameters in a study of the perturbations that the causative meteor streams suffer during their lifetime in the Solar System. Meteor streams are moved by the gravitational attractions of the major planets and obviously can be detected from Earth only when the stream crosses the Earth's orbit.

These ancient records also provide a fascinating insight into the interests and priorities of the astronomers of the time. Meteors were regarded as ominous omens, but due to their brief duration they were only minor omens in comparison with solar eclipses and comets. A great shower in March 763 was interpreted as 'a forecast of the end of the world'. On 17 October, 855 meteors shot westward across the sky all night and 'for this reason Emperor Hlotarius prayed, neglecting all his affairs'. Man's great desire to link cause and effect is illustrated by the account of the fireball seen on 11 August, 1353 which left behind an ash-coloured vapour, twisted like a snake. The chronicler stresses that 'after its appearance it did not rain for three months'. On 14 December, 1482, a very bright meteor was seen again in Parma, 'flying in a clear sky, and without noise—but there was an earthquake'.

It is also heartening to realise that meteorites were treasured in those days, just as they are now. One that fell in Catalogne during 1348 was sent to the King on the back of a mule. A meteorite as large as the head of a man fell near Altesleibon in Germany in 1136, and 'the brothers of the abbey kept it carefully'. We read of a

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meteorite which fell in 586 'roaring with sparkles'. But my favourite account is of a rather unusual 'lapis mirae magnitudinis'. 'A stone as large as a house fell near Duringia, Germany in 1135, with a terrible noise that lasted for three days. The lower part of it, about half, sank into the ground, burning for three days like steel from the fire'. Also on July 26, 1243 'so many stars fell that if they had been true stars none would have remained in the sky'.

It is apparent that, in addition to their scientific worth, these historical papers are a source of some marvellous quotations. □

Man and desert

from Peter D. Moore

THE problems associated with the spread of deserts are both social and ecological. The involvement and interest of man in the process of desertification made it an appropriate subject for consideration at the Tenth International Congress of Anthropological and Ethnological Sciences*. It is also appropriate that it should have formed the theme of a post-plenary session entitled, 'Anthropology and Desertification' and have been held at the Central Arid Zone Research Institute (CAZRI), Jodhpur, for this institution has contributed much to the understanding of, and attack upon, desertification in the Rajasthan Desert of north-west India.

Complex historical and economic factors have contributed to human mismanagement of marginal arid lands. The indigenous peoples of the Rajasthan Desert, according to H. S. Mann and S. P. Malhotra (CAZRI), have many traditional techniques for coping with semi-arid conditions, including mixed farming, fragmented land holdings (of advantage in scattered rainfall areas), fallow and rotational farming systems, migration in famine, as well as socioreligious customs and taboos, particularly in relation to the care of trees. Modern desertification problems in the area may well be due to the imposition of new pressures rather than the long-term persistence of old ones.

A similar note, although with an economic overtone, was struck by B. Spooner (University of Pennsylvania). The modern economic system operates at a level greater than that of the ecosystem. Market forces are now the chief determinants in societies

where once kinship and local organisation were most influential. Such a situation will encourage short-term exploitation of the local resources. Equally, the operation of rational, political processes at the new level may appear quite irrational at the ecosystem level. For this reason, even when ecological solutions to desertification problems can be envisaged, they may be difficult to implement locally. Historically, arid lands have often been politically independent and socially there may be resistance to innovative ideas from outside. It is vital, therefore, that the formulation of environmental policy should receive contributions from the indigenous society.

A novel and valuable feature of this conference was the involvement of anthropologists, environmental scientists and pastoral economists. The result was an unusual, yet productive, juxtaposition of disciplines and ideas. Among the pastoral economists, the contribution of S. Sandford (Overseas Development Institute, UK) is worth particular mention. He described pastoral systems as being either conservative or opportunistic. In the former, the number of grazing animals is kept at a constant, fairly low level. Since in most semi-arid areas there is considerable fluctuation in rainfall (and, therefore, in forage production) from year to year, the conservative system of pastoralism will keep overgrazing to a minimum, but will fail to exploit all of the available forage in a good year. An opportunistic system, on the other hand, varies the number of animals maintained according to the conditions prevailing. This should provide greater production efficiency, but in practice the number of stock is often reduced too little and too late, with consequent overgrazing and starvation. Environmentalists therefore tend to favour conservative strategies, but one is faced with the question of just how high the stock density should be set. In other words, how frequently can one tolerate the temporary over-exploitation of resources? If the answer is very infrequently, then stock density must be kept low, much of the resource is under-exploited and the entire system becomes far less attractive to the pastoralist than an efficiently managed, opportunistic system.

Specialised field studies and case histories in semi-arid areas are contributing much to understanding local problems. Several papers derived from a multi-disciplinary study of the Turan Biosphere Reserve of north-eastern Iran, a project directed by Spooner. C.

Hamlin (University of Pennsylvania) described the use of satellite remote sensing for the detection of changes in vegetation and sand distribution. A. E. Nyerges has examined the feeding behaviour of sheep and goats, which are herded in mixed flocks in the Turan. Adult goats proceeded at a rate of 25 m min⁻¹ whilst feeding and this allowed them to range further from villages when foraging than sheep, which proceeded at 17 m min⁻¹. Kids achieved only 14 m min⁻¹. The result of these different speeds is that a zonation of grazing pressures exists around villages and pens, in which distant areas are predominantly grazed by goats. The selectivity of the two animals also varies, for example, *Artemisia* comprises 23% of the sheep's diet, but only 15% of the goat's intake. Thus, both qualitative and quantitative variations in vegetation predation exist at different distances from a village.

A further pressure on vegetation associated with human settlement is the demand for fuel wood. M. Martin has estimated that a family requires 150 kg wood each month. The processing of milk products from a flock of goats



A hundred years ago

In connection with our article last week on a proposed Scottish observatory, it may be interesting to state that one day last August Mr. Milne Home, chairman of the Meteorological Society for Scotland, accompanied by Mr. Colin Livingston, headmaster, Public School, Fort William; Mr. Thompson, student; and Mr. David Doig, contractor, ascended Ben Nevis and made several observations with the view of erecting a station on the summit. They found the top enveloped in a mantle of snow—a circumstance which rendered it an extremely difficult task to select suitable spots for the erection of a dwelling-house and observatory. After a careful survey Mr. Home came to the conclusion that the plateau immediately beyond the spring affords the best site. The recommendation this spot has is its contiguity to the water-supply. It is proposed to construct the buildings after the following plan: first, a wall of stone with an inside lining of wood and an inner coating of felting, and the outside of the wall to be covered with corrugated iron. An external wall of stone would also be erected to serve as a protection from the blast. The estimated cost of the structure is 500l.

From *Nature* 19, 23 January, 279; 1879.

*Held in Delhi on 12–18 December, 1978. It was convened by B. Spooner (University of Pennsylvania) and H. S. Mann (Director of CAZRI).

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and sheep may require as much as 100–150 kg each day. Thus the total pressure is considerable in a habitat where, according to R. Bhadresa (King's College, London) the woody biomass above ground is only about 2,600 kg ha⁻¹.

One conclusion which emerges from an interdisciplinary study of this sort is that the ecological questions, such as those concerning the extent of human impact, are relatively easy to answer compared with the social problems which are likely to arise if attempts are made to reduce this impact. □

Galaxy clustering and anti-clustering

from William C. Saslaw

THE positions of galaxies on the sky contain much important astrophysical information. First, their spatial distribution provides a link with the conditions of their formation and the large scale expansion of the Universe. Second, their distribution may show evidence for haloes of absorbing gas or dust around galaxies. Interesting developments are occurring in both these areas.

In the standard view of our expanding Universe, galaxies formed at a redshift $Z \gtrsim 10^3$, after matter and radiation decoupled. We do not understand the detailed manner of their formation, nor do we know their initial distribution in space. Having formed, however, galaxies interact gravitationally as massive point particles and we can follow their changing distribution from some assumed initial state. The encouraging result is that the simplest initial state—a uniform random distribution—can lead to the main properties of the non-uniform clustering we now see.

This clustering of galaxies was first observed by Herschel and analysed by von Humboldt (*Cosmos*, 1866) even before they knew the nature of the nebulae. More recently, one of the most useful measures of clustering has turned out to be the two-point galaxy correlation function, $\xi(r)$, discovered by Totsuji and Kihara (*Pub. Ast. Soc. Japan* **21**, 221; 1969) who analysed the Lick survey of galaxies brighter than 19th magnitude. They found that the

difference between the observed probability of finding a galaxy at distance r from any arbitrary galaxy and this probability in a uniform random distribution, varies approximately as $r^{-1.8}$ over distances between about 0.5 and 10 megaparsecs. Their results have since been confirmed and extended by many astronomers, especially Peebles and his collaborators at Princeton.

To help understand these observations, several theoreticians working mainly with N-body computer codes developed by Aarseth at Cambridge have simulated the clustering of point masses in expanding universes. One of the major questions is to discover what ranges of initial clustering spectra and expansion rates are compatible with the observed two-point and higher order correlations. A forthcoming paper by Efstathiou (*Mon. Not. R. Astr. Soc.* **186**, 133; 1979) helps to clarify this question. He computed two 1,000-body clustering problems. Both started from exactly the same Poisson initial distribution. One evolved in a closed Einstein–de Sitter universe (which has density parameter $\Omega=1$). The other evolved in a more rapidly expanding open universe which started with $\Omega = 0.775$ and ended with $\Omega = 0.1$ after the expansion radius had increased by a factor of 31. The galaxies in the closed universe evolved into a distribution $\xi(r) \sim r^{-1.8}$ after the universe expanded to several times its original radius. This agrees with several previous simulations by others. The galaxies in the open universe, however, had a somewhat steeper correlation function at each stage of its expansion. Thus at any given chronological time, the expansion rate seems to be mirrored in the galaxy correlations.

Taken at face value, this would support the view that we live in a closed universe. However, as Efstathiou recognises, this would be a premature conclusion. One reason is that fluctuations of ~ 0.2 in the exponent of $\xi(r)$ are found among different 1,000-body simulations using the same initial statistical (but not identical) distribution. To reduce these 'ensemble fluctuations' one needs to use more particles. At present it is possible to compute $\sim 4,000$ -body problems in reasonable time using direct integration methods. There are plans to evolve about 20,000 bodies using Fourier techniques under conditions suitable for finding two-particle correlation functions, and these should produce results soon. Another difficulty is to sort out the results of different initial distributions of galaxies. Here one especially wants to learn whether the initial distributions which would evolve into the observed distribution using a rapidly expanding open universe need to be

very *ad hoc*. In the more distant future, enough galaxy redshifts could be measured to make use of constraints from velocity correlations as well as spatial correlations.

We turn now to a second aspect of the galaxy distribution: anti-clustering. Unlike the positive correlations just discussed which are found using a huge sample of galaxies, anti-clustering may be found among small subsets. These subsets make a negligible contribution to the overall correlation function, but are of special interest on their own. In particular, if there is a relative deficit of distant galaxies around nearby ones, the most plausible explanation would be an obscuring halo of gas or dust around each of the nearby galaxies. Such haloes, with a mass at least several times the mass in the easily visible body of the galaxy, have become fashionable in recent years as a means of stabilising spiral galaxies against the formation of a bar in their centre. It is now doubtful whether such a massive halo is really necessary for stability. This stability can also be provided by counterstreaming stars (Kalnajs, *Astrophys. J.* **212**, 637; 1977; Zang & Hohl *Astrophys. J.* **226**, 521; 1978) which remain from mergers which formed the galaxy, or by a concentration of mass in the nucleus of a galaxy. Moreover, it is not clear that the non-linear results of instability to bar formation, possibly followed by a transition to spiral or elliptical structure in an explosion or gravitational encounter, are inconsistent with observations. Nevertheless, the existence of galactic haloes remains an important question. For example, if most galaxies have haloes of about 10 times their presently observed mass, it would strengthen the evidence that we live in a closed universe.

Direct evidence for haloes could come from faint optical or X-ray emission around galaxies, from the rotational velocity of neutral hydrogen clouds around galaxies, and from gas or dust around nearby galaxies which absorbs the light of more distant galaxies. Observations with the first two techniques suggest that haloes do exist around some galaxies, but their masses are very uncertain. In the case of optical or X-ray emission we do not know the mass/luminosity ratio. This ratio depends on both the composition and geometric distribution of material in the halo. With neutral hydrogen clouds, the masses can be determined more accurately but the observations are not easy to make. They suggest haloes of moderate mass.

The third technique, galaxy anti-clustering, has not been pursued vigorously. This is because, until very recently, there was not a large enough

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complete sample of galaxies with known distances in which to look for statistical anti-clustering between near and distant galaxies. The relative distances had to be estimated from rather fickle secondary indicators such as apparent magnitude or size. Using these secondary indicators, Bogart and Wagoner (*Astrophys. J.* **181**, 609; 1973) found weak evidence for anti-clustering between near and distant rich clusters of galaxies, and Wesson (*Astrophys. Lett.* **19**, 127; 1978) has claimed to find this effect among 274 bright galaxies in the local supercluster. Now, however, many more galaxy redshifts are becoming available and they will enable much better analyses of anti-clustering based on distance to be made. Sandage (*Astrophys. J.* **83**, 904; 1978) has published

719 redshifts for galaxies in the local supercluster, and more are to come. Recently Vera Rubin of the Carnegie Institute in Washington DC has compiled a list of lists of redshifts published in 1976–1978 and of measurements in progress. More than 5,500 redshifts were published in this period and a roughly equal number should be measured in the next year or two. This will be a sample to conjure with. In addition, powerful new machines, such as the one which Kibblewhite and his associates are completing in Cambridge, will determine the positions and characteristics of galaxies on photographic plates rapidly and automatically. Thus in the years just ahead we can expect to understand much more about the distribution of matter over the largest scales of the Universe.

relativistic effects, the most exciting of which is the confirmation of the existence of gravitational waves. He announced the measurement of the advance of periastron (similar to the excess advance of the perihelion of Mercury attributed to relativistic effects) at an earlier Texas conference. Refinements in the measuring equipment and techniques have allowed Taylor, and his colleagues L. Fowler and P. McCulloch, to improve the timing accuracy of the pulse arrivals by a factor of 20. An iterative method has then been used to obtain corrections up to the third order in (v/c) .

In particular, the rate of decrease of the orbital period is observed to be $\sim -3.2 \times 10^{-12} \text{ s s}^{-1}$, and is of the correct sense and magnitude to agree with the general relativity prediction of energy and angular momentum loss by way of gravitational radiation. The importance of gravitational radiation in causing the spiralling together of binary companions has long been realised in the evolution of close non-pulsar binary system. This is the first time that a quantitative measurement has been made. The binary pulsar is apparently turning out to be a remarkably clean laboratory for relativistic astrophysics. Further work, especially theoretical, will, it is hoped, check all other possible causes of the observed effects and refine the measurements already made. The current data restrict the masses of the pulsar and its companion to around $1.4 M_{\odot}$ each, consistent with a pair of neutron stars, or a neutron star (the pulsar) and a white dwarf. Changes in the observed pulse shape hint at spin-orbit coupling causing the pulsar spin axis to process.

J. Nelson (Lawrence Berkeley Laboratory) reported on an optical object found by Crane within 0.3 arcsecs of the position of the binary pulsar. If it is the pulsar, then the pulsed fraction is less than 1%, and if it is the companion then it must be hotter than $\sim 18,000 \text{ K}$ in order to be able to radiate the observed flux without causing an excessive periastron advance.

The discovery of the most unusual stellar spectrum of 1978 was undoubtedly reported by B. Margon (University of California, Los Angeles). The 14th magnitude emission line object numbered 433 in the catalogue of Stephenson and Sanduleak shows emission lines that move across the spectrum! These strong features have been seen to shift by up to $600 \text{ \AA}$ (an equivalent velocity shift of $\sim 0.1 c$) in 30 days. SS433 has been the subject of intense study recently since it was

Gravitational waves confirmed at Texas Symposium

from M. C. Begelman, A. C. Fabian, N. A. Sharp and A. Wakelin

THE peripatetic Texas Symposium on Relativistic Astrophysics went to Munich in 1978* with, for the first time, participants from the People's Republic of China. Traditionally, the symposium is not regarded primarily as a forum for announcing the latest results, but more as a chance to review the progress of the past 2 years. With several exceptions—notably the new findings on the binary pulsar and the discovery of a new type of emission-line object—this meeting largely followed suit.

The first day was devoted to extragalactic sources, which, to the relativistic astrophysicists, meant quasars, BL Lacertae objects, and Seyferts. There was little theoretical debate, black holes and beams are seemingly well entrenched as the current fashion, and most of the reported progress was observational. It seems that the defining characteristics of the various types of object are still controversial. Several speakers (and recent observations) suggested that common classification schemes, based on spectral signature, may be misleading. For instance, it seems that all Seyferts may radiate the bulk of their energy in X rays, and, so far, there are three known X-ray quasars. Infrared also figured strongly, but sadly there was no discussion of the wealth of ultraviolet data now available from the IUE satellite.

Of particular cosmological interest

was the report on the microwave background, devoted mainly to the very careful work of D. P. Woody and P. L. Richards of the University of California Lawrence Berkeley Laboratory. The spectrum now appears to be only approximately black body with a temperature of $\sim 3 \text{ K}$, being instead too peaked and too narrow. This deviation cannot be explained by any of the processes currently expected to produce deviations. Further theoretical work and further observations will doubtless be available by the next Texas meeting.

There was also a very lucid review by G. F. R. Ellis (University of Capetown) of the many assumptions and corrections which are made by cosmologists when considering the available data. Although much of this is known to the expert practitioner it deserves a wider audience amongst all people collecting and interpreting data of cosmological interest.

It was not until halfway through the conference that classical relativity theory made its appearance, with a clear discussion by J. Ehlers (Munich) of the difficulties of treating isolated systems. This is of great importance in the interpretation of the binary pulsar.

The common meeting ground for relativists and astronomers is this binary pulsar. Discovered in 1974 by Hulse and Taylor, the pulsar swoops around its companion in a highly elliptical orbit at velocities $\sim 0.1\%$ of the velocity of light. J. Taylor (University of Massachusetts at Amherst) reported the detection of four more

*The 9th Texas Symposium on Relativistic Astrophysics was held in Munich on 14–19 December, 1978.

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realised that it was associated with a variable radio source (see for example Clark & Murdin, *Nature* **276**, 44; 1978; Ryle *et al.* *Nature* **276**, 571; 1978). An X-ray source A1909+04 is also in the right position and it is located in line with the supernova remnant W50. Margon and his colleagues are the first to observe the unusual optical emission features. Two in particular lie either side of H α , the infrared one of which moved to longer wavelengths by 150 Å, the red feature moving in the opposite sense by 70 Å, in just four nights observing. SS433 presents a challenge; perhaps it too will become a regular at the Texas conferences. □

Pathogenic mechanisms of mycoplasmas

from D. B. Archer

MYCOPLASMAS are a diverse group of wall-less prokaryotes. They have been implicated as possible causal agents in a multitude of diseases in animals, plants and insects but have been demonstrated to be causative in relatively few instances and in only a handful of cases is there any information which bears directly on the pathogenic mechanisms involved. However, from the Proceedings of the 2nd Conference of the International Organisation for Mycoplasmaology (IOM) (*Zentralbl. Bakt. Parasitenk. Infekt. Hyg.* **241**; 1978) it is clear that an understanding of the pathogenic mechanisms is now being worked towards.

The diversity in form of mycoplasmas reflects the range of diseases and mechanisms involved. Some mycoplasmas are motile and motility may be necessary for these organisms to reach their target site, although a non-motile variant of *Spiroplasma citri* is known to be pathogenic when injected into host phloem cells by an insect vector (Townsend *et al.* *J. gen. Microbiol.* **100**, 15; 1977). Mycoplasmas lack flagella and their mechanisms of motility are poorly understood although an actin-like protein has been reported in *Mycoplasma pneumoniae* (Neimark *Proc. natn. Acad. Sci. U.S.A.* **74**, 4041; 1977). However, Rodwell *et al.* (IOM Proceedings) showed

that no mycoplasma protein co-electrophoresed with rabbit muscle actin by two-dimensional gel electrophoresis. Although Rodwell *et al.* could not detect a mycoplasma protein having a high affinity for deoxyribonuclease 1, a characteristic of eukaryotic actin, Kahane *et al.* (IOM Proceedings) were able to isolate a multiprotein complex having a high affinity for muscle myosin.

Attachment to host cells is of primary importance in the pathogenicity of *Mycoplasma gallisepticum* and *M. pneumoniae*, among others. A *M. pneumoniae* membrane protein (P1) is required for the attachment to host tracheal cells (Hu *et al.* *J. exp. Med.* **145**, 1328; 1977) and although these workers could not detect any staining of *M. pneumoniae* proteins with PAS, a carbohydrate stain, it is of interest to know whether P1 is identical to the recently isolated glycoprotein from *M. pneumoniae* (Kahane & Brunner *Infect. Immun.* **18**, 273; 1977) because carbohydrate has been implicated in the attachment of this organism to host epithelium (Powell *et al.* *Infect. Immun.* **13**, 959; 1976). Apart from the presence of a glycoprotein in *M. pneumoniae*, and possibly *M. gallisepticum* (Goel & Lemcke *Ann. Microbiol.* **126**, 299; 1975) there are no confirmed reports of glycoproteins in any other mycoplasmas. If carbohydrate is important in the host cell attachment of other mycoplasmas it need not be through a glycoprotein and Huang (*Nature* **276**, 624; 1978) has recently pointed out that glycolipids can be involved in cell adhesion, although glycolipids do not seem to be involved in the host cell adhesion of *M. pneumoniae* (Hu *et al. op. cit.*). Both *M. pneumoniae* and *M. gallisepticum* adhere to erythrocytes and this attachment has been used as a convenient model for natural host cell attachment. It seems that glycophorin is the major erythrocyte receptor for *M. gallisepticum* (Banai *et al.* IOM Proceedings; *Infect. Immun.* **21**, 365; 1978) whereas this is not the case with *M. pneumoniae* (Feldner *et al.* IOM Proceedings), although bindings of *M. pneumoniae* to host epithelial cells is reduced by pre-treating host cells with neuraminidase (Powell *et al.* *Infect. Immun.* **13**, 959; 1976; Gabridge, IOM Proceedings). These mycoplasmas also bind to glass and plastic so it is of interest to know what determines specificity of binding *in vivo*. Sialic acid is of importance in the binding of *M. gallisepticum* to erythrocytes but this residue alone probably could not provide specificity of host. Isolation of the presumed binding proteins of mycoplasmas and determination of their affinities for target sites would enable some judge-

ments to be made on the specificity of mycoplasma-host cell interactions.

Host cell attachment may not be as important in determining specificity as the production of a mycoplasma toxic substance or susceptibility of the host to it. Indeed, there is no evidence for high affinity host cell attachment by most mycoplasmas. Unfortunately, information on toxic products of mycoplasmas is meagre. Hydrogen peroxide and ammonia are produced by some mycoplasmas and may be toxic factors, particularly if the association of the mycoplasma with the host is intimate (for example *M. pneumoniae*) or if the amount of hydrogen peroxide is large (*Mycoplasma mycoides* subsp. *capri*). Specificity may more easily be explained by the production of a toxic compound which has selectivity of action, but little information on such substances is available. Characterised toxic cell products are the galactan of *M. mycoides* subsp. *mycoides* (Lloyd *et al.* *J. med. Microbiol.* **4**, 425; 1971; Buttery *et al.* *J. med. Microbiol.* **9**, 379; 1976) and the exotoxin of *Mycoplasma neurolyticum* (Tully, INSERM **33**, 317; 1974). Also, low molecular weight toxins have been isolated from *S. citri* (Daniels & Meddins INSERM **33**, 195; 1974; Daniels IOM Proceedings). One hopes that purification and characterisation of such toxic products will lead to experiments designed to determine their modes of action. Exotoxins were not detected, however, in culture filtrates of *M. gallisepticum*, an organism known to be neurotoxic. (Thomas *Ann. N.Y. Acad. Sci.* **143**, 218; 1967). Some mycoplasmas bind to and transform lymphocytes (Ginsburg & Nicolet *Nature new Biol.* **246**, 143; 1973; Cole *et al.* IOM Proceedings; Wise *et al.* IOM Proceedings) and form caps (Stanbridge IOM Proceedings; Stanbridge & Weiss *Nature* **276**, 583; 1978) demonstrating mitogenic activity *in vitro*. Also, Cole *et al.* (IOM Proceedings) reported that mycoplasmas induce the release of cytotoxic factors from lymphocytes. The *in vivo* importance of mycoplasma interactions with lymphocytes is unknown but provides a plausible explanation for many of the effects of mycoplasma infection.

In conclusion, although information on the pathogenic mechanisms of mycoplasmas is still limited the situation is improving. Specificity remains a major problem which can only be overcome by characterisation of the cell residues involved in host cell attachment of those mycoplasmas which do attach, and by isolation and characterisation of toxic factors. Demonstration of *in vivo* mitogenicity would clearly be a major advance in our understanding of the pathogenic mechanisms of mycoplasmas. □

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articles

Lowland Maya radiocarbon dates and the classic Maya collapse

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Three processes of the Late Classic Maya collapse, the demise of the stela cult, the breakdown of elite culture, and the reduction of the commoner population, are compared by frequency-distribution bar-graphs of dated monuments in the Southern Lowlands and radiocarbon dates from elite and commoner contexts. The demise of the stela cult and the breakdown of elite culture seem to have coincided. The commoner radiocarbon bar-graph does not necessarily indicate the occurrence of a major demographic catastrophe.

THE Late Classic Maya collapse poses one of the most challenging problems in world archaeology. Briefly, it represents an abrupt cessation of Lowland Maya elite class culture—particularly such activities as the use and production of carved and dated monuments, platform-temples, elaborate residential structures, fine polychrome pottery, and carved jade—during the ninth and early tenth centuries AD. The demise of the elite class was apparently accompanied by a rapid and large-scale depopulation of the commoner class, particularly in the Southern Lowlands (eastern Chiapas, the Peten and western Belize).

Our study is intended not to explain the events of the collapse, but to empirically measure the rate of decline in several cultural processes integral to the collapse. Three phenomena of particular interest are the cessation of the 'stela cult'; the decline in the general activities of the elite class; and the apparent population reduction of the commoner or peasant farmer class. For each of these cases we have produced a 'decline curve' that is based on a frequency-distribution bar-graph of relevant chronometric data, namely the dedicatory Long Count dates inscribed on monuments, and radiocarbon dates from elite or commoner contexts.

Traditional theories have tried to explain the collapse from either an internal or external orientation^{1,2}. The internal theories emphasise either natural catastrophes such as overpopulation, soil exhaustion, disease, earthquakes, hurricanes, climatic change, and insect pests; or socio-political crises such as peasant revolts and intersite warfare. The external theories view the collapse in terms of cultural developments, such as military or economic events, throughout the whole of Mesoamerica. Unfortunately, the majority of these hypotheses are speculative single-cause theories which are not supported by statistically valid empirical data.

A new research phase on the Maya collapse was initiated in 1970 at a seminar by the School of American Research, Santa Fe, New Mexico³. The theoretical model, summarised by Willey and Shimkin⁴, is tailored to future hypothesis-testing. This model states that certain activities of the elite class which had successfully stimulated the growth of Early Classic Maya

civilisation became inappropriate for a new Late Classic society faced with a burgeoning population, precarious agricultural production, and military harassment by central Mexican groups. The cumulative effect of these disrupting factors was to administer a fatal shock to the Maya political system. The demise of the elite class is thought to have been marked by the erection of the last calendrically dated stelae at Seibal and Uaxactun in AD 889 or in Tonina in AD 909; the termination of monumental architecture at Tikal in AD 830, Altar in AD 909 and Seibal in AD 928; and the appearance of the Tepeu 3 ceramic horizon in the ninth century AD that signalled the end of the Peten polychrome tradition and the appearance of foreign fine paste wares. Following the tenth century no general recovery of Classic Maya civilisation took place in the Southern Lowlands. Some pockets of elite tradition continued to exist, however, along with reduced commoner populations, at such sites as Tikal, Altar and Seibal⁴.

Analysis of stela dates

The Santa Fe model relies heavily on stela dates to construct a chronology for the collapse of the Maya elite. Yet it fails to provide a comprehensive frequency-distribution of Lowland stela erection. This is done in the following section, as a test of the Santa Fe model.

The erection of stelae and altars was an important activity for the Classic Maya. The labour necessary for the erection of these multi-ton limestone monuments ensured that the stela cult crosscut Maya religion, politics and economics. The quarrying and sculpting, for example, was probably done by professional masons and artisans. Many dozens, and sometimes several hundreds, of labourers had to drag the megalith to the site. The average Peten stela had a mass of about 5 metric tonnes, while the largest in the Lowlands (stela E at Quirigua) had a mass close to 50 tonnes⁵. The elite no doubt maintained a high visibility as the organisers of this impressive operation—because precisely such displays of power helped to maintain their authority. The upright rectangular stela, featuring a life-size low-relief portrait of the ruler, often colourfully painted, had a hieroglyphic text with such information as the ruler's genealogy and the dates of his accession to the throne, marriages, battles, and so on. Smaller cylindrical altars, usually with a shorter text and less sculptural ornamentation, were placed in front of the stela. In addition to their role as a power displays for the elite, the stelae and altars also had important religious functions. They were usually erected on 'period-endings' that commemorated important calendric periods such as Katuns and Hotuns (9.9 yr). Their hieroglyphic texts contain substantial technical information on calendars and other ritualistic activities. Finally, ceremonial caches were frequently buried underneath or alongside the stela. These monuments clearly served as foci for both political and religious activities.

The number of dated Maya monuments, both stelae and altars, erected in the Southern Lowlands per Katun cycle (19.8 yr) during the period AD 292–909 is shown in Fig. 2. The span of time ranges from the erection of the earliest long count-dated stela yet found in the Lowlands (Tikal St 29) at AD 292, up to the last securely-dated Lowland stela at Tonina. Graphs similar to Fig. 2 have been previously published^{6–8} but to our knowledge Fig. 2 is the most comprehensive for the Southern Lowlands. It includes 415 Long Count dates, most of them period-endings, marked on 359 stelae and 56 altars from 62 sites. The boundary for the Southern Lowlands is marked in Fig. 1. Southern Lowland sites from which we obtained either stelae or altar dates for our study are marked in Fig. 1 by a triangle, while sites that furnished radiocarbon dates are marked by a star. The area that we have marked as the Southern Lowlands is almost identical to that used by the Sante Fe Seminar³. (The stelae data⁵

were obtained from the literature and also from personal communication with J. Graham and I. Graham.)

Recent evidence suggests the possibility that Protoclassic rulers (AD 100–250) of Highland Guatemalan centres, such as Kaminaljuyu, exported the stela cult to the Lowlands by military conquest or marriage alliance⁹. Figure 2 shows that few dated monuments were erected in the Lowlands before AD 475 (9.2.0.0.0.). Sites with these early monuments are clustered within a 30 km radius in the north-east Peten. Following the major waterways, however, the stela cult moved rapidly into neighbouring regions and by AD 534 (9.5.0.0.0.) the maximum geographical spread for Classic Maya stelae had been reached¹⁰. Shortly afterwards the growth of the cult was slowed by a brief and enigmatic hiatus that lasted from AD 534–613 (9.5.0.0.0.–9.9.0.0.0.) which apparently was caused by the withdrawal of Central Mexican political influence in the Peten¹¹.

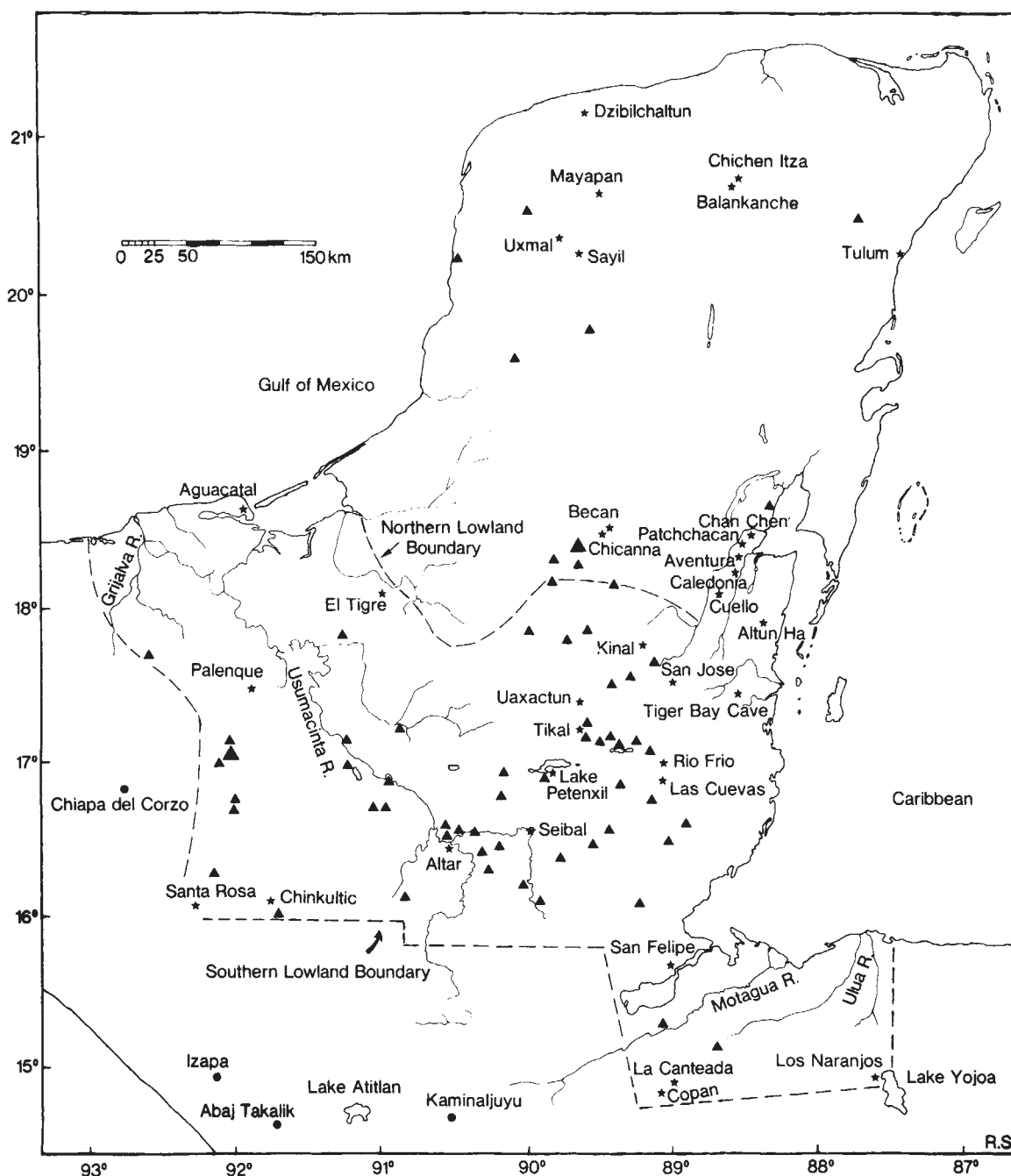


Fig. 1 Map of Maya Lowlands showing sites with dated monuments and radiocarbon dates. Only Southern Lowland sites were used in study. Note both long count dates and radiocarbon dates were used from Tikal, Uaxactun, Palenque, Seibal, Altar and Copan. The last Terminal Classic stelae were erected at Tonina, Chiapas and San Lorenzo, Campeche (sites marked by large triangles).

The explosive growth of the cult during the period AD 613–790 (9.9.0.0.0.–9.18.0.0.0.) is clear in Fig. 2. During this period at least 324 stelae and altars were dedicated in both the Southern and Northern Lowlands. The climax of the stela cult was reached in the eighth century. This zenith can be measured by several statistical conventions. The mean stela date, calculated from a sample of 300 stelae, is AD 705 (ref. 5). The date AD 731 (9.15.0.0.0.) is the statistical mode, as it occurs on more monuments than any other period-ending¹², while the date AD 790 was commemorated by at least 19 ceremonial centres, more so than any other period-ending^{6,7}.

The popularity of the cult took a sudden downwards plunge at AD 810 (9.19.0.0.0.). Its rapid decline culminated with the erection of the last securely-dated stela at Tonina, Chiapas in AD 909 (10.4.0.0.0.) (I. Graham, personal communication). We include in Fig. 2 an even later stela at San Lorenzo, Campeche which has been dated by J. E. S. Thompson to AD 928 (10.5.0.0.0.)¹³. Its absolute date cannot be established conclusively, however, as it bears only a floating Calendar Round date.

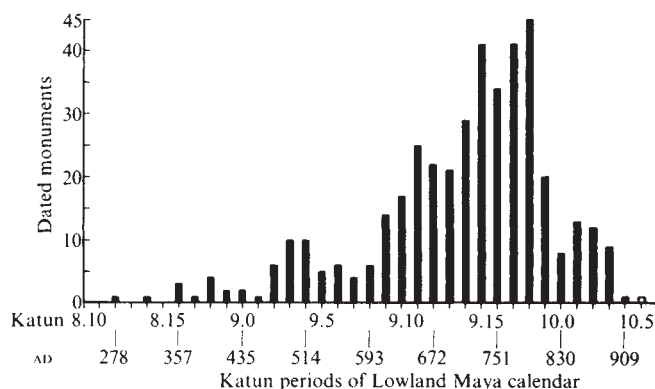


Fig. 2 The number of dated monuments erected per Katun period in the Southern Maya Lowlands. All monuments that were erected during a given Katun period are indicated in the position directly above the start of the Katun. Included are: 415 LC dates carved on 359 stelae and 56 altars from 62 Southern Lowland sites.

Figure 2 shows that a genuine collapse or abrupt breakdown of the stela cult occurred, rather than a gradual or normal decline in popularity, by both the asymmetry of the distribution and the steep downslope. The growth and maturity of the cult lasted some 500 yr, whereas its decline took place in about a century. This sudden loss of an established tradition of ruler glorification does favour the demise of the rulers themselves and their entourage.

Bar-graphs of radiocarbon dates

In their model Willey and Shimkin⁴ found that the degree of commoner population decline following the loss of the elite was the most formidable research problem of the Maya collapse. They did not specify the exact degree to which populations of the Southern Maya Lowlands either died out or migrated. Adams, on the other hand, apparently relying on ceramic evidence, has estimated that an unusually rapid depopulation from 3,000,000 to 450,000 took place in the countryside and at ceremonial centres in a period from 75 to 100 yr (ref. 14). Similarly, Culbert offers a population loss for the Lowlands in excess of a million people within a century and describes it as one of the world's greatest demographic disasters.

In his synthesis of ceramic chronologies for the Santa Fe Seminar, Rands demonstrated that in general the average Maya ceramic period lasted 75 yr, with an uncertainty of ± 30 yr for either the beginning or ending date⁸. Thus ceramically-based population studies at any given Maya site can only suggest that a house mound had been occupied during part or all of a 75 yr period. A further complication is the problem of the terminal stratigraphic layer, which in many cases represents the transitional Late Classic–early Postclassic occupation of the site.

This stratum is particularly troublesome because it lacks a bordering stratum that can fix an incontrovertible upper temporal limit³.

Frequency-distribution bar-graphs of Lowland Maya radiocarbon dates offer an alternate or supplementary dating method necessary in establishing rates of decline for non-stelae dated Late Classic elite activities and the relative population size of the commoner class. A large number of Maya radiocarbon dates already exist, and many others could be readily obtained from the hundreds of skeletons and charcoal samples that have been collected and stored during major Maya field projects by Canadian, American, Mexican and Guatemalan academic institutions. The average range of uncertainty associated with Maya radiocarbon dates so far collected is of the order of ± 80 yr, which is similar to the time span of the average Maya ceramic phase.

To determine whether elite activities in general did undergo a radical decline at the same time as the collapse of the stela cult, we have produced a frequency-distribution bar-graph of 96 radiocarbon dates whose archaeological context represents various Southern Lowland Maya elite activities. Figure 3a shows the frequency of these activities through the period 51 BC to AD 1389. These activities, as dated by the ^{14}C method, include the inhabitation of palaces, the building of platform-temples and palaces; the production of sophisticated carved wooden lintels for palaces and temples; the production of elegant wooden figurines and bark-paper codices; and the administration of cave ceremonies and elaborate burials.

Most of the 153 radiocarbon dates in Table 1 have already been published and were compiled through an intensive search of the literature. Table 1 also includes 22 previously unpublished ^{14}C dates from Tikal, Guatemala, that were measured by the University of Pennsylvania Radiocarbon Laboratory (W. Coe and E. Ralph, personal communication). Table 1 is intended to be a comprehensive list of most of the ^{14}C dates presently available from the Maya Lowlands during the period 51 BC to AD 1389. Accordingly, it contains an additional 27 dates from the Maya Northern Lowlands that are not plotted in Fig. 3a, which are marked by an asterisk. Table 1 gives the archaeological provenience of the ^{14}C sample and classifies this provenience into an elite or commoner context. The classification was made by us on the basis of the excavator's description of the excavation context. All radiocarbon dates, whether previously published or not, were converted to tree-ring calibrated dates using the chart of Suess^{16,17}. For purposes of standardisation the calibrated date was rounded to the nearest interval of 50. Accordingly our calibrated date may differ slightly from previously published versions. Any other

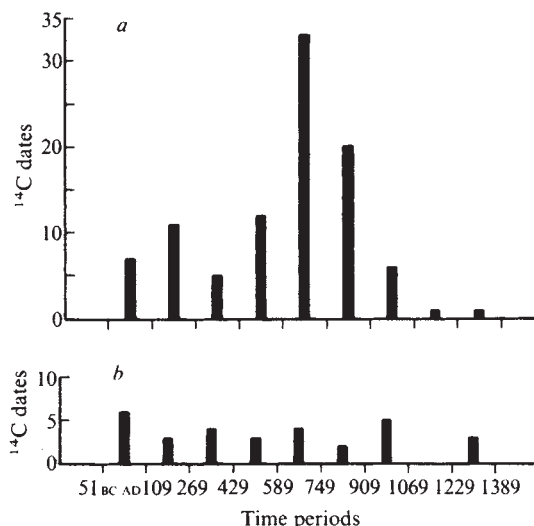


Fig. 3 a, Radiocarbon dates from elite contexts in the Southern Lowlands per time period; b, Radiocarbon dates from commoner contexts in the Southern Lowlands per time period.

calibration can be based on the BP radiocarbon date in Table 1. For some samples we have assigned a time range or several date possibilities instead of a single date. This situation exists because the calibration curve is not always singularly definitive. In plotting these cases on Fig. 3a we used either the midpoint of the range or the average of alternate dates. Table 1 also specifies the material composition of the radiocarbon sample, the laboratory number of the processed sample and the published reference.

Figure 3a indicates that Maya elite activities are present throughout the time span 51 BC to AD 589 (the Protoclassic and Early Classic periods) and their relative frequency shows only minor fluctuations. Elite activities dramatically increase during the period AD 589–749, and attain their zenith during this point. A significant decrease of these activities is observed during the period AD 749–909, followed by a drastic decline that takes place from AD 909–1069. Maya elite culture in the Lowlands during the period AD 1069–1389 is almost negligible.

The asymmetry and steep downslope in Fig. 3a clearly demonstrate a breakdown of elite class culture during the tenth century in the Lowlands, and its cessation, for all practical purposes, by AD 1069. The decline curves presented in Figs 2 and 3a are remarkably similar. A minor difference is that elite

culture declines somewhat later than does the stela cult, a lag of the order of about one century. This was also noted by Hammond at Pusilha, Belize¹⁸. However, as Fig. 3a involves a broader spectrum of individuals and activities than Fig. 2, this time lag is expected. It is reasonable to conclude that the graphs in Figs 2 and 3a are measuring somewhat different aspects of the same phenomena—the rise and fall of the Classic Lowland Maya elite class. The similarity of the two graphs helps to establish the reliability of the ¹⁴C frequency-distribution bar-graph method.

As previously discussed the depopulation rate of the Lowland centres, following the collapse of elite culture, is the most difficult problem of the Maya collapse. Figure 3b shows the distribution frequency of 30 ¹⁴C dates, within the span 51 BC to AD 1389, that are from what may be called Southern Lowland Maya commoner contexts. In other words, they are not inherently associated with elite manifestations. The ¹⁴C samples come from commoner contexts that include house mounds, burials, middens, ridged fields, and lake sediment deposits which contain maize pollen as evidence of nearby agriculture.

It is immediately apparent that Fig. 3b indicates mildly fluctuating population cycles in the Lowlands, but no radical growth spurts or drastic declines. A slight population decline

Table 1 Lowland Maya radiocarbon dates for the span 51 BC to AD 1389

Mexico	Site	Provenience	Context (elite or commoner)	Tree-ring calibrated date† (AD)	Radiocarbon date† (yr BP)	Material	Laboratory no.	Ref.
Mexico	Aguacatal	Pit JA-1	C	300	1,645 ± 140	Charcoal	I-1068	22
		Tr. B-6, Level II	C	300	1,695 ± 200	Animal bone	I-1069	22
		Tr. B-6, Level III	C	700, 800, 900	1,212 ± 170	Animal bone	I-1070	22
	Balankanche Cave*	Chamber 2 urn	E	900–1,000	1,072 ± 51	Charcoal	P-1132	23
		Chamber 2 hearth	E	1,000	1,028 ± 42	Charcoal	P-1133	23
	Batchatón*	Urn cremation	E	1,450	410 ± 70	Charcoal	GRN-790	24
	Becan*	Str. IV, Rm. 9	E	700, 800–900	1,230 ± 95	Charcoal	I-4286	25
		Str. IV, Rm. 8	E	650	1,295 ± 95	Charcoal	I-4287	25
		Str. XXV	E	650	1,280 ± 95	Wood	I-5028	25
		Str. XXV-sub	E	150	1,830 ± 95	Charcoal	I-5029	25
	Chicanna*	Str. X	E	650	1,345 ± 95	Wood	I-5030	25
		Str. II, Rm. 1	E	650	1,280 ± 95	Charcoal	I-5024	25
		Str. II, Rm. 1	E	650	1,265 ± 95	Wood	I-5025	25
		Str. XX	E	650	1,380 ± 95	Wood	I-5026	25
	Chichen Itza*	Str. XX	E	700, 800–900	1,230 ± 95	Wood	I-5028	25
		Chultun by Cenote, Secondary burials	E	900	1,135 ± 60	Bark	UCLA-1706	—
		La Iglesia	E	900	1,170 ± 70	Wood	TBN-313-1	26
		La Iglesia	E	600	1,350 ± 70	Wood	TBN-313-2	26
	Chinkultic	Red house	E	600	1,340 ± 60	Wood	TBN-313-3	26
		El Castillo lintel	E	900	1,160 ± 70	Wood	Y-626	27
	Dzibilchaltun*	Temple-platform tomb	E	1,000	990 ± 70	Bone collagen	UCLA-1617	—
		Str. 1 lintel	E	500–550	1,450 ± 200	Wood	W-707	28
	El Tigre	Str. 612 hearth	C(?)	500	1,520 ± 200	Charcoal	LJ-531	28
		Ridged field	C	350	1,670 ± 49	Wood	P-1625	29
Guatemala	Mayapan*	Str. Q-162	E	1,250	700 ± 95	Charcoal	GRO-452	30
		Str. R-87	E(?)	1,450	400 ± 55	Burnt wood	GRO-1166	31
	Palenque	South of palace	E	650	1,390 ± 100	Charcoal	H-396	32
		Above bedrock, Ex. 23	E(?)	550–650	1,440 ± 100	Charcoal	H-397	32
		Ofrenda, 2, T-V, N. group	E	450	1,565 ± 105	Charcoal	H-639	32
		North side of palace	E	450	1,540 ± 105	Charcoal	H-641	32
	Sta. Rosa	Md. B	E	150	1,845 ± 50	Charcoal	GRN-1916	24
		Md. F, Fl. 3	C	100 BC–AD 50	1,990 ± 65	Charcoal	GRN-1932	24
	Sayil*	Main palace	E	950	1,100 ± 70	Wood	Wis-144	33
		Main palace	E	700, 800–900	1,230 ± 60	Wood	IVIC-484	34
Guatemala	Tulum*	Str. 21, Rms. D, E	E	1,150–1,250	880 ± 60	Wood	Y-393	27
	Uxmal*	Structure behind magician pyramid	E	700, 800, 900	1,210 ± 60	Wood	IVIC-485	34
		Magician pyramid	E	650	1,390 ± 50	Wood	Y-627	27
	Chiapas/North Yucatan?	Nunnery quadrangle	E	900–1,000	1,065 ± 100	Wood	GRO-613	30
		Grolier codex	E	1,250	720 ± 130	Bark paper	I-6107	35
	Mexico–Guatemala?	Sculpture of Maya noble	E	550–650	1,425 ± 120	Wood	I-490	36
Guatemala	Altar de Sacrificios	House mound 33	C	350	1,290 ± 155	Charcoal	GX-166	37
		Str. A-II, fill	E	100 BC–AD 50	1,990 ± 130	Charcoal	GX-169	37
		Str. A-III, burial 4	E	150	1,845 ± 110	Charcoal	GX-168	37
		Str. B-II	E	50	1,965 ± 225	Charred beans	GX-171	37
	Kinal	Str. A-XIX, refuse	E	650	1,275 ± 115	Charcoal	GX-170	37
		Main plaza, E. temple	E	650	1,320 ± 73	Wood	UM-124	38
	Lake Petenxil	Pollen core from under 4 m of water	C	650	1,305 ± 140	Gyttja	Y-988	39
		Pollen core from under 4 m of water	C	1,000	1,040 ± 200	Gyttja	Y-1289	39
	San Felipe	Pollen core from under 4 m of water	C	1,350	635 ± 140	Gyttja	Y-987	39
		Pit F	C	50 BC–AD 50	1,970 ± 100	Charcoal	Y-2416	40
	Seibal	Md. II, Cut 8	C	650	1,310 ± 100	Charcoal	Y-2418	40
		Md. II, Cut 6	C	550–650	1,430 ± 100	Charcoal	Y-2419	40
		Burial 19	E	500	1,490 ± 320	Collagen	UCLA-1640D	41
		Burial 17	E	550–650	1,420 ± 95	Collagen	UCLA-1640C	41
	Tikal	Mass burial 4	E	950	1,100 ± 100	Burnt bone	UCLA-1640F	41
		Burial 12	C	1,000	1,000 ± 65	Collagen	UCLA-1640A	41
		Burial 13	C	1,050	940 ± 70	Collagen	UCLA-1640B	41
	Tikal	T-II	E	650	1,365 ± 50	Wood	P-960A	—
		T-II	E	450	1,605 ± 51	Wood	P-961	—
		T-III V.B.	E	650	1,358 ± 39	Wood	P-949	—
		T-III V.B.	E	650	1,352 ± 45	Wood	P-950	—
		T-V	E	650	1,267 ± 51	Wood	P-947	—
		T-V	E	650	1,317 ± 51	Wood	P-948	—

Table 1 Lowland Maya radiocarbon dates for the span 51 BC to AD 1389 (cont.)

Site	Provenience	Context (elite or commoner)	Tree-ring calibrated date† (AD)	Radiocarbon date‡ (yr BP)	Material	Laboratory no.	Ref.
	T-VI B	E	500	1,527 ± 51	Wood	P-962	—
	T-VI B	E	650	1,297 ± 44	Wood	P-963	—
	T-VI B	E	650	1,364 ± 50	Wood	P-1196	—
	T-VI B	E	1,000	999 ± 44	Wood	P-1197	—
	T-VI B	E	650	1,372 ± 49	Wood	P-1198	—
	T-VI B	E	650	1,308 ± 47	Wood	P-1199	—
	T-VI B	E	900	1,125 ± 46	Wood	P-1200	—
	T-VI B	E	650	1,398 ± 42	Wood	P-1201	—
	Str. 3D-43	E	650	1,290 ± 47	Wood	P-964	—
	Str. 3D-43	E	500	1,544 ± 41	Wood	P-965	—
	Str. 5D-46	E	250	1,718 ± 45	Wood	P-946	—
	Str. 5D-46	E	450	1,580 ± 50	Wood	P-955	—
	Str. 5D-46	E	650	1,376 ± 53	Wood	P-956	—
	Str. 5D-46	E	200	1,789 ± 33	Wood	P-957	—
	Str. 5D-46	E	450	1,555 ± 46	Wood	P-958	—
	Str. 5D-46	E	300	1,677 ± 46	Wood	P-959-A	—
	Str. 5D-52, Rm. A, V.B. 8	E	650	1,335 ± 40	Wood	UCLA-158A	42
	Str. 5D-52, Rm. A, V.B. 9	E	650	1,304 ± 60	Wood	UCLA-158B	42
	Str. 5D-52, Rm. A, V.B. 10	E	650	1,366 ± 60	Wood	UCLA-158C	42
	Str. 5D-52, L. 34, a	E	650	1,318 ± 60	Wood	UCLA-158D	42
	Str. 5D-52, L. 34, b	E	650	1,303 ± 50	Wood	UCLA-158E	42
	Str. 5D-23, V.B. 4	E	650	1,350 ± 52	Wood	P-294	43
	Str. 5D-23, V.B. 8	E	250	1,740 ± 50	Wood	P-295	43
	Str. 5D-23, V.B. 14	E	250	1,720 ± 61	Wood	P-296	43
	T-IV Rm. 2, V.B. 1	E	900	1,150 ± 44	Wood	P-235	43
	T-IV Rm. 2, V.B. 2	E	700, 800–850	1,250 ± 48	Wood	P-236	43
	T-IV Rm. 2, V.B. 3	E	700, 800–850	1,260 ± 72	Wood	P-237	43
	T-IV Rm. 2, V.B. 4	E	700, 800, 900	1,200 ± 48	Wood	P-238	43
	T-IV Rm. 3, V.B. 1	E	700, 800–900	1,230 ± 48	Wood	P-242	43
	T-IV Rm. 3, V.B. 2	E	700, 800, 900	1,220 ± 55	Wood	P-243	43
	T-IV Rm. 3, V.B. 3	E	700, 800, 900	1,210 ± 83	Wood	P-244	43
	T-IV Rm. 3, V.B. 4	E	700–750, 900	1,180 ± 79	Wood	P-245	43
	T-IV L. 3, a, d, or f	E	700, 800, 900	1,190 ± 47	Wood	P-248	43
	T-IV L. 3, e or g	E	700, 800, 900	1,210 ± 48	Wood	P-249	43
	T-I Rm. 1, V.B. 1	E	500	1,490 ± 85	Wood	P-229	43
	T-I Rm. 1, V.B. 2	E	650	1,300 ± 93	Wood	P-230	43
	T-I Rm. 2, V.B. 2	E	700, 800–850	1,260 ± 49	Wood	P-232	43
	T-I Rm. 3, V.B. 2	E	700, 800, 850	1,260 ± 56	Wood	P-234	43
	T-I L. 3, c or d	E	650	1,270 ± 49	Wood	P-247	43
	T-I L. 3, e	E	700, 800–850	1,260 ± 110	Wood	P-251	43
	Str. 5D-34, Rm. 3, C-19	E	900	1,150 ± 47	Charcoal	P-278	43
	Str. 5D-34, Rm. 3	E	900	1,180 ± 55	Charcoal	P-279	43
	Str. 5D-34, Rm. 3, Altar	E	500	1,530 ± 52	Charcoal	P-281	43
	Str. 5D-34, Construction fill	E	450	1,550 ± 58	Charcoal	P-284	43
	Great Plaza Chultun	E	50 BC–AD 50	1,970 ± 44	Charcoal	P-285	43
	Quarry 5D-1	C(?)	100 BC–AD 50	2,000 ± 63	Charcoal	P-287	43
	N. Acropolis earth layer fill	C	50	1,930 ± 51	Charcoal	P-757	44
	N. Acropolis, Burial 85	E	50	1,934 ± 63	Wood	P-535	44
	N. Acropolis, Str. Sub. 2-1st	E	50	1,951 ± 46	Charcoal	P-561	44
	N. Acropolis, in situ pit	E	100	1,874 ± 54	Charcoal	P-562	44
	N. Acropolis, Ceremonial debris	E	50	1,926 ± 48	Sherds, ash	P-565	44
	N. Acropolis, Burial 125	E	250	1,777 ± 45	Charcoal	P-768	44
	N. Acropolis, Str. 5D-26-1st	E	400	1,625 ± 60	Wood	P-571	44
	N. Acropolis, Str. 5D-20-2nd	E	650	1,308 ± 49	Charcoal	P-569	44
	N. Acropolis, Str. 5D-20-1st	E	650	1,274 ± 50	Wood	P-570	44
	N. Acropolis, Str. 5D-26-1st	E	400	1,624 ± 61	Charcoal, sherds	P-572	44
	N. Acropolis, Burial 22	E	550, 650	1,416 ± 52	Charcoal	P-573	44
	N. Acropolis, Str. 5D-26-1st	E	650	1,285 ± 52	Charcoal	P-574	44
	N. Acropolis, Hearth	E	250	1,749 ± 59	Charcoal, bone	P-575	44
	T-IV L. 3, left plank	E	700, 800–850	1,250 ± 50	Wood	UCLA-1373A	45
	T-IV L. 3, 3rd plank from left	E	650	1,370 ± 50	Wood	UCLA-1373B	45
	T-IV L. 3, right plank	E	650	1,400 ± 60	Wood	UCLA-1373C	45
Uaxactun	Str. A-VIII, Rm. 8	E	250	1,780 ± 60	Wood	Y-367	27
	Str. A-V, Rms. 36, 54	E	650	1,330 ± 70	Wood	Y-368	27
Belize							
Altun Ha	Str. E-1 tomb	E	500	1,544 ± 62	Carbonized copal?	P-1028	44
Aventura	Ex. 2-3, Midden	C	500–550	1,450 ± 40	Charcoal	UCLA-1921E	5
	Ex. 2-3, Midden	C	1,250	680 ± 80	Shell	UCLA-1938E	5
	Ex. 1-1, Midden	C	650	1,400 ± 80	Shell	UCLA-1938B	5
	Ex. 3-2, Midden	C	1,000	1,025 ± 80	Shell	UCLA-1938F	5
Caledonia	Ex. 20-13, Midden	E	300	1,680 ± 70	Charcoal	UCLA-1921F	5
Chan Chen	Ex. 24-3, Habitation soil	C	100	1,865 ± 55	Charcoal	UCLA-1921B	5
	Ex. 30-5, Str. B-3	E	300–450	1,580 ± 60	Charcoal	UCLA-1921C	5
	Ex. 28-2, Construction fill of Sr. F-1	E	200–300	1,770 ± 50	Charcoal	UCLA-1921D	5
Cuello	Op. 17B, Level 23	C	250	1,700 ± 60	Burnt wood	UCLA-1985D	46
	Op. 17B, Levels, 20, 22	C	900	1,140 ± 100	Burnt wood	UCLA-1985BC	46
	Op. 17C, Level 12	C	1,050	930 ± 85	Burnt wood	UCLA-1985F	46
Las Cuevas	Outer case, Tr. 7	E	1,050	920 ± 150	Charcoal	BM-37	47
Patchchacan	Ex. 14-2, Burial	C	1,250	760 ± 110	Collagen	UCLA-1938C	5
Rio Frio Cave	West side of far cave	E	900	1,120 ± 150	Charcoal	BM-67	48
San Jose	Main Palace	E	1,000	1,010 ± 50	Wood	UM-122	38
Tiger Bay Cave	Upper chamber, torch	E	1,200	780 ± 60	Wood	UCLA-1935A	—
Honduras							
Copan	Hieroglyphic stairway construction fill	E	250	1,700 ± 110	Charcoal	UCLA-1419	49
	Hieroglyphic stairway	E	700, 800, 900	1,200 ± 70	Charcoal	UCLA-1420	49
La Canteada	Plaza 1, Pit 6	C(?)	250	1,700 ± 125	Burnt wood	UCLA-1992B	—
	Plaza 2, Str. 5, Pit 7	E	900–1,000	1,050 ± 220	Burnt wood	UCLA-1992C	—
Los Naranjos	Habitation soil	C	100	1,850 ± 100	Soil	GIF-1324	50
	Habitation soil, 2.3 m depth	C	250	1,700 ± 100	Soil	GIF-1473	50
	Habitation site, hearth	C	500	1,530 ± 100	Soil	GIF-1472	50
	Habitation soil	C	500	1,500 ± 100	Soil	GIF-1474	50
	Soil of ceremonial area	E	700, 800–850	1,260 ± 90	Soil	GIF-1326	50

* The radiocarbon date is not plotted in Fig. 3a, b, either because it is in the Northern Lowlands or it falls outside the range 51 BC to AD 1389.

† Date is rounded off to nearest 50.

‡ Half life 5,568 ± 30 yr.

might be indicated for the period AD 1069–1389, as only three ^{14}C dates are present, but given the overall small sample size this is hardly evidence of a major demographic catastrophe for this period. Figure 3b does not establish, then, a depopulation curve similar to those previously demonstrated for the cessation of the stela cult and general elite activities during the Late Classic–Terminal Classic periods.

The lack of a collapse curve in Fig. 3b may be due to the relatively small sample size or perhaps indicate a historical fact. In particular, the sample used is three times smaller than that in Fig. 3a and about 12 times as small as that in Fig. 2. A further complication is the geographical context of the ^{14}C samples. Only 35% of the dates come from the Peten, while the rest are from adjacent areas such as Belize, Chiapas, and Honduras, areas that may have been the scene of a delayed collapse⁴. Unfortunately the 30 dates represent most, if not all, of the ^{14}C dates presently available from Lowland commoner contexts. We therefore urge Maya archaeologists to obtain more commoner-associated ^{14}C dates, as these dates should eventually be of great value in resolving the depopulation issue and be more representative of the cultural situation as it actually was. Yet we also wonder if any major depopulation would not have been apparent in the 30 dates obtained so far.

A critical reexamination of the depopulation problem has in fact been offered by J. E. S. Thompson¹⁹ who concluded that

wholesale desertion of the Central area at the close of the Classic period can be ruled out. He proposed that a Postclassic population, probably reduced in size, had continued to exist in the Central Lowlands in isolated villages. This population was depopulated, in turn, during the first 50 yr of contact by Europeans, due largely to newly-introduced diseases such as dysentery, hookworm, and malaria. Additional support for a rethinking of the depopulation issue is found in Hellmuth's bibliography of the post-Conquest Lowland Maya² and in a recent article by Adams and Smith that compares the extensive population decline in mediaeval Europe to that of the Late Classic Maya²¹.

The decline curves for the cessation of the stela cult and the breakdown of general Late Classic elite activities seem to be very similar, although the latter process continued for another century following the demise of the stela cult. The ^{14}C bar-graph that simulates the population level of the commoner class during this period does not, however, indicate the occurrence of a major demographic catastrophe.

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Normal human tissues contain RNA and antigens related to infectious adenovirus type 2

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Normal human tissues have been found to contain RNA which hybridises with four regions of the adenovirus type 2 genome, including one which contains the transforming gene(s) of this virus. The RNAs have also been extracted from gorilla organs, but not from those of chickens, suggesting that they are a feature of all normal higher primate tissues. Serological tests suggest that these homologous RNAs are translated into proteins connected with essentially normal and virus-infected cell functions.

THE adenoviruses^{1,2} are associated with respiratory infections in humans. Many serotypes of these viruses can induce tumours in rodents^{3–6}, and adenovirus type 5 transforms human cells in culture⁷. During studies on human cancer, we have investigated the possibility that human tissues might contain RNA sequences related to human DNA viruses, using the technique of *in situ* hybridisation^{8–10}. We have found that normal human tissues contain RNA that hybridises with four regions of the genome of adenovirus type 2 (Ad-2). One of these regions includes the transforming gene(s) of this virus^{7,11,12}. This suggests either that genes similar to viral transformation genes are present in man in

much the same way that the avian *sarc* genes are homologous in base sequence to the *src* (transforming) gene of the Rous sarcoma virus^{13,14}, or that infectiously acquired Ad-2 DNA can persist under host control. The human cells expressing the RNAs in greatest abundance seem to be solitary, possibly circulating cells. Human tissue cultured cells apparently do not contain an abundance of these RNAs, which may account for the fact that they have not been described previously.

Hybridisation studies

In looking for possible RNA sequences related to Ad-2 viral DNA in uninfected human organs, we initially used placenta as the RNA source because it is available in quantity. Moreover, fetal tissues are often associated with endogenous viral sequence expression¹⁵⁻¹⁷. Total RNA was extracted by standard procedures¹⁸ and iodinated¹⁹ with ¹²⁵I for use in hybridisation. Equal amounts of Ad-2, human DNA and λ phage DNA or Ad-12 DNA were denatured and spotted on nitrocellulose strips and hybridised by the usual procedure²⁰ (for details see legend to Fig. 1). *Escherichia coli* total ¹²⁵I-labelled RNA served as an RNA control, hybridised to a parallel series of spots but substituting *E. coli* DNA for phage DNA. The hybridised strips were then treated with RNase and, after suitable washing, were dried and autoradiographed on presensitised X-ray film (Royal X-omat XHI Kodak) at -70 °C in cassettes fitted with intensifying screens. Such spot tests are extremely sensitive and the amount of signal due to hybridisation or to background is immediately visually apparent. Hybridisation can be quantitated if necessary by cutting out the spots and scintillation counting. This procedure was followed as described later to construct melting-temperature curves of the hybrids.

After autoradiographic exposure, there was no trace of hybridisation to phage DNA. In fact, the spots containing this DNA were, if anything, below background radioactivity levels in some cases, suggesting that the bound DNA may have pre-empted potential binding sites for molecules that contribute background. There was, as expected, very strong hybridisation to human DNA. Ad-2 DNA showed moderate hybridisation (Fig. 1a), which easily reached autoradiographic saturation on prolonged exposure (Fig. 1b). Ad-12 DNA showed a barely detectable autoradiograph (not shown). *E. coli* ¹²⁵I-labelled RNA did not hybridise with any of these DNAs, but did so with *E. coli* DNA.

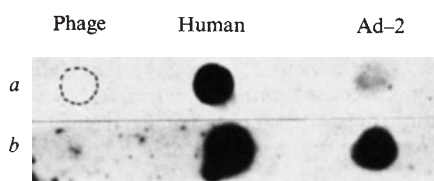


Fig. 1 Spot tests of hybridisation of ¹²⁵I-labelled human placental RNA to DNA of λ phage, human and adenovirus type 2 (Ad-2). The location of the negative hybridisation is shown by a dotted circle. Hybridisation has occurred to human DNA and to a lesser, but definite, extent to Ad-2 DNA. The conditions for hybridisation were as follows. The DNA samples were brought to the same concentrations, and 1- μ g aliquots bound in spots to a strip of nitrocellulose filter by the method of Gillespie and Spiegelman²⁰. The filter strip was then incubated for 6 h at 68 °C in 6 $\times$ SSC containing 0.02% each of Ficoll, bovine serum albumin (BSA) and polyvinyl pyrrolidone (PVP). It was then transferred to 3.6 ml of this same solution containing 2 mM EDTA, 0.5% SDS and $\sim 7 \times 10^7$ c.p.m. of placental total RNA previously prepared¹⁸ and radiolabelled¹⁹ with ¹²⁵I. The mixture was annealed at 68 °C for 15-16 h, cooled rapidly and the filter strip washed in 2 $\times$ SSC, treated with RNase (40 μ g ml⁻¹, 1 h, 37 °C in 2 $\times$ SSC), washed in 2 $\times$ SSC, treated with pronase (400 μ g ml⁻¹ in 2 $\times$ SSC, 1 h), incubated in 2 $\times$ SSC, 0.5% SDS for 6 h at 68 °C, washed exhaustively in a large volume of 2 $\times$ SSC, dried in a vacuum oven and autoradiographed for (a) 10 d, (b) 3 weeks at -70 °C using Kodak Royal X-omat XHI X-ray film presensitised by brief exposure to light from a calibrated masked flash gun.

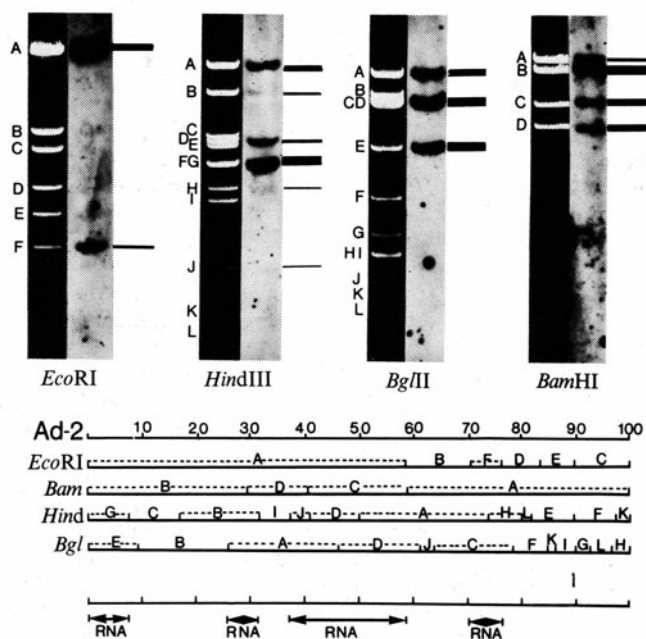


Fig. 2 Restriction digests of Ad-2 separated into fragment classes by electrophoresis on agarose gels stained with ethidium bromide and photographed in ultraviolet light, are shown paired with autoradiographs of ¹²⁵I-labelled placental RNA hybridised to the same fragments after transfer to nitrocellulose filter membrane and hybridisation as detailed in the legend to Fig. 1, and in the text. The bands in the digests are identified by letters to the left of each gel track. For technical reasons, the smallest restriction fragments do not photograph satisfactorily, and some of the minor autoradiographic bands are difficult to detect consistently together in the same autoradiograph. Because of this we have included a third track which indicates, diagrammatically, for each enzyme digest, all the bands to which we have obtained hybridisation in replicate experiments. These data are also shown below the photographs in the form of maps of the positions of the restriction fragments obtained with each enzyme in relation to the Ad-2 DNA map, which is divided into 100 fractions, according to the usual convention. The restriction fragments which hybridise with placental RNA are indicated for each digest by dotted lines over the fragment concerned. From these data, the provisional assignments have been made for the maximum limits to the Ad-2 genome sequences which hybridise with human total RNA; these are represented as double-ended arrows below the relevant locations. The conditions for electrophoresis were 1% agarose gels run at 40 V for 10 h, or 80 V for 4 h, in a buffer containing 15 mM Tris, 18 mM NaH₂PO₄, 0.5 mM EDTA, pH 7.8.

These results indicated that Ad-2 DNA contained some sequences homologous with human RNA, although they did not show whether these might have arisen from possible contaminating host cell sequences. Nevertheless, the fact that Ad-2 and Ad-12 were grown in similar human cells but behaved differently on hybridisation with human RNA suggested that contamination was unlikely. To investigate this point, we hybridised the same DNAs after digestion with the restriction endonuclease *EcoRI*, which cuts λ and adenovirus DNAs into small numbers of fragments; these can then be separated on agarose gels and transferred to nitrocellulose strips by the Southern procedure²¹ for subsequent hybridisation with the same ¹²⁵I-labelled human RNA probe. After autoradiographic exposure there was hybridisation only to Ad-2 DNA and this was limited to the *EcoRI* A and F fragments (Fig. 2). Separate lots of Ad-2 DNA were then cut with three further restriction endonucleases, *BamHI*, *BglII* and *HindIII*, which are known to produce genomic fragments overlapping and different from those produced by *EcoRI*. Hybridisation of human placental ¹²⁵I-RNA to these sets of fragments, as before, yielded the following hybridisation data (Fig. 2).

The fragments that hybridised were *EcoRI* A and F; *HindIII* A, B, D, FG, J and H (the latter two very faintly); *BglII* A, CD and E; and *BamHI* A, B, C and D. These data define the following provisional maximum limits for the hybridising regions. As only the A and F fragments of *EcoRI* hybridised, all the observed hybridisation must lie within these limits. As *EcoRI* A, *BamHI* B, *HindIII* G and *BglII* E fragments hybridised, but *HindIII* C or *BglII* B did not, the left end of *HindIII* C defines the most rightward limit of the first region of hybridisation at 7.5 units on the Ad-2 map²². This region, from 0 to 7.5, is known to contain the transforming gene(s)²³. Because the *BglII* B fragment did not hybridise, but the *BglII* A fragment did, the latter, with the other restriction enzyme fragments that overlap it, defines the beginning (left end) of the next region of hybridisation at approximately 26 units. *HindIII* I did not hybridise but *HindIII* J did, so the I fragment defines a non-hybridising gap, and the left end of J defines the beginning of the next region of hybridisation at approximately 37 map units, extending to approximately 58.5 units, which is defined by the left end of *EcoRI* B, which did not hybridise. By the same reasoning the *EcoRI* F fragment must define the limits of the fourth region of homology as between approximately 70.7 and 75.9 map units. From this analysis, we presume that the doublet *HindIII* FG concerns hybridisation only to the G fragment, but that hybridisation occurs to both fragments of the *BglII* CD doublet. This point can be finally decided when a more complete analysis of the hybridisation patterns has been carried out. This provisional map summarising the maximum limits within which the hybridising regions lie is given diagrammatically in Fig. 2.

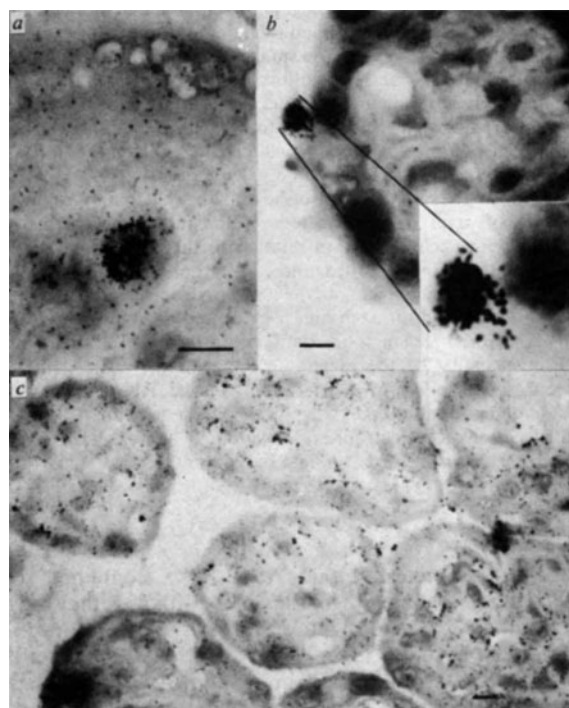


Fig. 3 Autoradiographs showing concentrations of Ad-2 complementary RNA in placenta. Ad-2 DNA labelled by the 'nick-translation' reaction with ^3H , was denatured and hybridised *in situ* to frozen sectioned human placenta in conditions that detect complementary cellular RNA⁸⁻¹⁰. *a*, A 'solitary' cell that shows a high concentration of label signifying a high concentration of Ad-2-related RNA. This cell type occurs relatively infrequently (about 2-3 per $\times 40$ objective field) and occasionally seems to be loosely attached to the tissue (*b*). This may reflect the fact that it is a circulating cell. *c*, An example of a generally labelled area of placenta, after hybridisation as above. The grain distribution in this autoradiograph suggests that whole areas of this tissue contain Ad-2-related RNA at a lower level than the solitary, highly labelled, cells shown in *a* and *b*. Autoradiographic exposure 3 weeks. Controls of similarly hybridised λ phage DNA were negative. Scale bars, 10 μm .

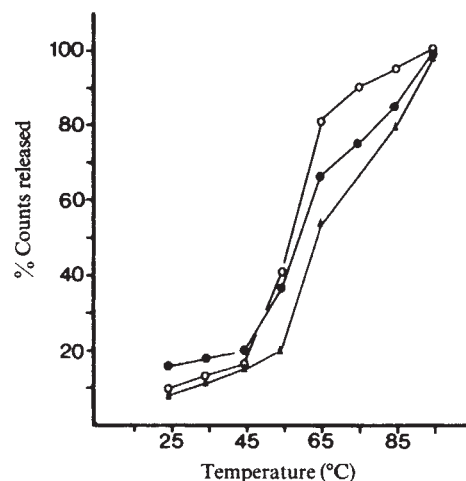


Fig. 4 Thermal dissociation of hybrids formed between human total ^{125}I -labelled RNA and human total DNA ($\circ$), and Ad-2 whole genomic DNA ($\bullet$, sample 1; $\blacktriangle$, sample 2), from different sources. The curves show similar features, with both Ad-2 DNA samples having formed hybrids of rather higher thermal stability than total human DNA. Hybridisation conditions are described in the legend to Fig. 1. Dissociation was carried out by cutting spots of hybridised DNA from nitrocellulose filters of spot tests that had been shown to be positive by previous autoradiography on X-ray film as in Fig. 1. These were then counted by scintillation spectrometry in toluene-based scintillation fluid, washed and then treated in diethyl pyrocarbonate 0.1 in SSC (0.15 M NaCl, 0.015 M sodium citrate, pH 7.4) for 30 min, washed in SSC, and heated for 10 min in 1 ml 0.1 SSC and 0.1% SDS at each of the temperatures shown. The counts released were recovered from solution by trichloroacetic acid precipitation in the presence of 100 μg BSA per sample, dried by suction filtration and counted by scintillation spectrometry. Approximately 80% of the original radioactivity was recovered.

This provisional map has been confirmed with a further two independently obtained samples of Ad-2 DNA and with 10 separate samples of placentas, obtained from normal births, from two hospitals. We have also found the same pattern in the case of human adult liver RNA, and with liver, kidney, brain and spleen RNA obtained from a gorilla which died from the effects of minor surgery. The RNAs from four separate human cell lines maintained in permanent culture gave very faint patterns of hybridisation compared with the organ RNAs and are being studied in more detail. RNA obtained from chickens in the same manner as for other organs of primates did not hybridise in two separate tests.

That human organ RNA and other higher primate RNA contain sequences with homology to certain regions of the genome of Ad-2, but that such RNA apparently is relatively much less abundant in cell lines of human origin, indicated that the distribution of cells having such an abundance was not uniform. We investigated this aspect by hybridising ^3H nick-translated, denatured Ad-2 DNA to human placental cryostat sections *in situ* using a method which permits hybridisation to mRNAs⁸⁻¹⁰. After S_1 nuclease treatment and thermal rinsing to remove unhybridised probe or poorly matched hybrids, the preparations were autoradiographed and examined microscopically. Strong hybridisation occurred only to certain cells in the sections. These seemed to be solitary cells, with hybridisation to almost the entire area of the cell (Fig. 3*a, b*). Other areas of the sections showed a lower level of hybridisation which was, however, above the non-cellular background (Fig. 3*c*). There was no hybridisation in the case of λ phage ^3H -DNA hybridised in identical conditions. We presume from this that the bulk of the RNA that hybridised is contributed by a tissue cell sub-population and that cells of this type are not among the cell lines that we examined.

Melting-temperature experiments

We carried out melting-temperature experiments to determine the thermal stability of the hybrids formed on nitrocellulose between Ad-2 DNA and placental RNA. These were done by cutting out the relevant hybridised spots from the filter and heating them in solution at increasing temperature (see legend to Fig. 4) to measure the loss of hybridised RNA from the filters. Hybrids formed between human ^{125}I -labelled total RNA and two separate Ad-2 DNA samples and with total human DNA were compared. As can be seen from Fig. 4, the melts of Ad-2 hybrids showed similar characteristics and similar, though slightly higher, T_m values compared to the homologous hybrids, indicating that the hybrids to Ad-2 were reasonably thermally stable. This result was expected from the annealing conditions (legend to Fig. 1) which were quite stringent and essentially identical to those used in most published Ad-2 hybridisation studies (see discussion). We conclude that the hybrids that we detect between human RNA and Ad-2 DNA are at least as well matched as those in previous transcription studies of Ad-2. We are now investigating methods to determine the precise lengths of the hybridised regions. However, this is complicated by the fact that the organ RNA is to some extent degraded.

Immunological studies

Hybridisation between a viral DNA and human RNA from apparently uninfected tissues suggests similar protein coding functions in the sequences concerned. To test this hypothesis, rabbit cells infected with a clinical isolate of Ad-2 were used to raise antisera separately against the early-infected and late-infected cells (see legend to Fig. 5). The antisera were then reacted with six human cell lines (not shown) in an indirect immunofluorescence assay. All except one cell type, a human glioma (NG-118H) transformed by Rous sarcoma virus which was faintly positive, were completely negative. Controls of Ad-2-infected HEP 2 cells were brilliantly positive (Fig. 5a) as were transformed rat cells containing the extreme left-hand fragment of the Ad-2 genome¹² (not shown). Placental sections were also positive when treated with the anti-early antisera (Fig. 5b,c), but not with anti-late-infected cell antiserum. The fluorescence was concentrated on the surfaces of cells lining what seemed to be channels in the tissue as well as occurring diffusely in the cytoplasm of many areas. Some cells showed the nuclear fluorescence usually seen in placental cells. However, this was rare and not intense. Of 10 placentas tested, 8 were undoubtedly positive in these tests and 2 were judged negative. We do not understand this variation, as all were positive in hybridisation tests for Ad-2-related RNA; however, it may be due to the fact that the antigens concerned are unstable and that the variations reflected the variable storage conditions of the tissues before we obtained them.

Antigens were then solubilised²⁴ from placental tissue and tested by gel diffusion in Ouchterlony plates against both early- and late-infected cell antisera, using controls of Ad-2-infected rabbit cell antigens (Fig. 6). There was no reaction with any dilution of uninfected cell antigens (not shown). Strong precipitin lines formed between the early-infected cell antisera and the antigens of both early- and late-infected cells, as expected, for both will contain the same early antigens, although presumably in different concentrations. Weaker but quite definite precipitin lines formed between this same antiserum and the wells containing placental antigens, and these showed partial identity with one of the lines originating from the early-infected cell antigen wells but no identity with late-infection antigens. The tests against anti-late-infected cell antisera were even more strongly positive against the infected cell antigens, particularly the late-infected cells. However, there were no visible precipitin lines between this antiserum and the wells containing placental antigens. Separate tests were then carried out in which a range of dilutions of the placental antigens were tested against each antiserum separately. No dilution used gave any precipitin lines with the late-infected cell antiserum (not shown). The early-

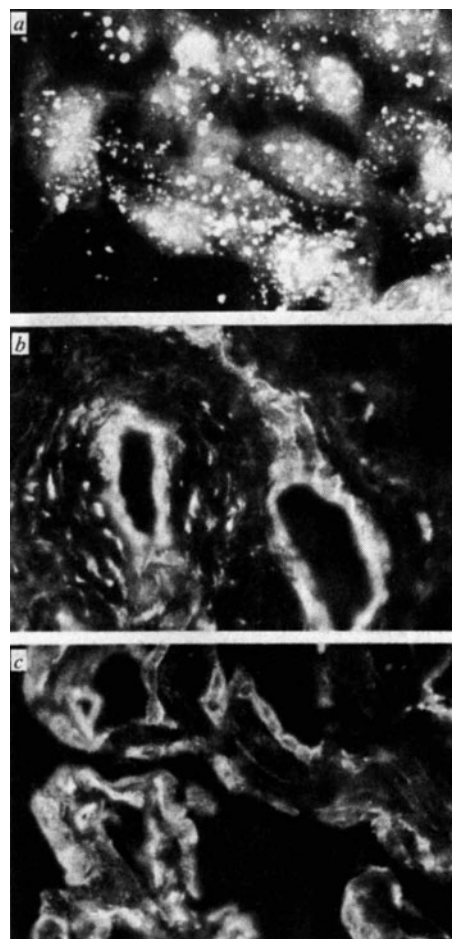


Fig. 5 Indirect immunofluorescence tests for antigens on: *a*, Ad-2 early-infected human cells (HEP 2); *b*, *c*, normal human placenta cryostat sections, using antiserum raised against Ad-2 early-infected rabbit cells (Rk-13), at a dilution of 1/40 and sheep anti-rabbit IgG conjugated with fluorescein isothiocyanate (Wellcome). The rabbit cells were grown in medium 199 supplemented with 2% rabbit serum and the antisera raised in rabbits. Cells were infected for 20 h in the presence of $20\ \mu\text{g}\ \text{ml}^{-1}$ cytosine arabinoside. The resulting antiserum was negative for virus neutralising activity. Uninfected cell antiserum was also raised and was negative by immunofluorescence with these same subjects, and infected cell antiserum was negative with both uninfected HEP and Rk-13 cells. The infecting adenovirus was obtained from a clinical isolate. Antiserum to late-infected cells was negative in similar tests. Fluorescence in the HEP cells is mainly on nuclear structures with some cytoplasmic fluorescence. Placental sections show stronger fluorescence in cytoplasmic regions, especially on what are apparently cells lining channels or vessels in the placenta, but nuclear fluorescence is also visible. These results, which demonstrate the presence of cross-reacting antigens between early-infected and apparently uninfected placenta, have also been obtained with all other tissues but not with cell lines tested (see text), and are confirmed by Ouchterlony gel diffusion (Fig. 6 and text).

infected cell antiserum, however, showed a single precipitin line that altered position between the antigen and antibody well depending on concentration. This shift is due to antigen-antibody concentration which affects the solubility and hence the mobility of the antigen-antibody complex. The fact that there was no precipitin line in the case of anti-late-infected cell antiserum suggests that the concentration of the relevant antibodies to early-infected cell antigens was very low.

We conclude from these preliminary immunological experiments that rabbit cells infected with Ad-2 contain certain antigens that are not present in uninfected rabbit, or human, cells in culture. We further conclude that some of the Ad-2-induced antigens are identical to tissue antigens present in most

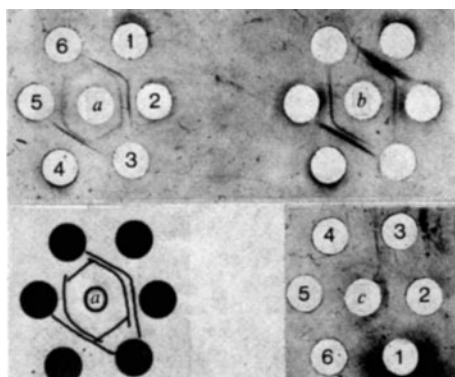


Fig. 6 Ouchterlony gels showing patterns of precipitation obtained between *a*, anti-early Ad-2-infected Rk-13 cells, and *b*, anti-late Ad-2-infected Rk-13 cell antisera. Wells are numbered 1–6 in the same manner in each case. Wells 1 and 4 contained dilutions of 1/50 and 1/100 of late Ad-2-infected antigens; 2 and 5 contained dilutions of 1/50 and 1/100 Ad-2 early infection antigens; 3 and 6 contained 1/50 and 1/100 dilutions of placental antigens. An interpretation of the pattern seen in *a* is shown below it. In *a*, precipitin lines can be seen between both wells containing placental antigens and the central well containing anti-Ad-2 early-infected Rk-13 cells. In 3 there is a suggestion of partial identity with one of the lines associated with well 2 as judged from the single spur. There are no lines visible between the central well and wells 3 and 6 in *b*, indicating absence from the late-infected cell of antigenic components related to those of normal placenta. Dilution experiments with this particular combination over the range of 1/10 to 1/1,000 of antigen concentration revealed no precipitation (not shown); however, a similar range of dilutions of the same combination in the case of *a* showed an arc of precipitation spiralling out from the centre well according to the dilution of antigen; this is shown in *c*, in which all wells contained placental antigen, as follows: (1) undiluted; (2) 1/10; (3) 1/50; (4) 1/100; (5) 1/1,000; (6) saline. These data confirm that normal human placentas express antigens that are associated with Ad-2 early infection as shown by immunofluorescence in Fig. 5.

human placenta samples, concerning mainly the early proteins of the adenovirus infectious cycle. Such antigens are not detectable on cell lines of human origin by immunofluorescence but were present in all tissues of gorilla (not shown). We are now attempting to characterise these antigens in more detail. The presence of antigens related to Ad-2 early function proteins in humans and in gorilla tissues correlates with the presence in these tissues of RNA molecules that hybridise with regions of the Ad-2 genome involved in coding early function proteins. We suppose from this that these RNAs code for the antigens that are shared between apparently normal human, and other primate, tissues. The *in situ* hybridisation result using Ad-2 DNA probes suggests that certain cells, or cells in a certain transitory state, which constitute a minor subpopulation in the placenta, contain a high concentration of the Ad-2-related RNA. The connection between this observation and the fact that the antigens concerned are rather ubiquitous in the tissues examined is unclear but is discussed below. However, as there are at least four regions of the Ad-2 genome that hybridise, it is possible that different cell types contain different proportions, or types, of the corresponding, homologous, RNAs, and consequently code for different antigenic moieties.

Discussion

The present findings are unique in the sense that there have been no previous reports of RNA sequences related to adenoviruses in apparently uninfected human cells. These findings are particularly intriguing as they relate to adenoviruses that are potent transforming viruses in animal hosts^{3–6}. Moreover, the regions of homology include sequences in the extreme left-hand end of the Ad-2 genome that contains transforming genes of this virus. The consistency of the findings, there being no negative results in the 11 human tissue samples included in this study, indicates

that the RNAs concerned may be ubiquitous in the population. Moreover, the presence of the same RNA molecules in gorilla is indicated from the similarity of the hybridisation patterns obtained with RNA from kidney, liver, spleen and brain. This suggests that the RNAs may also be common within the higher primate group, although more examples, involving some from a natural population, will be essential to support this.

Our criteria for hybridisation of the RNAs to Ad-2 DNA are those normally used in hybridisation mapping of Ad-2 (refs 25, 26) and are reasonably stringent taking the genome as a whole. Increasing the hybridisation temperature to 75°C reduced but did not prevent hybridisation. Moreover, the thermal stability of the Ad-2 DNA – human RNA hybrids seems representative of hybrids formed between human total DNA and the same human RNAs used in this work. We conclude from this that the hybrids are reasonably well matched. This is supported by the fact that there is demonstrable homology between the antigens coded during infection by Ad-2 and those present in human placenta and liver, and gorilla organs, which indicates similar coding functions of the RNA produced early in Ad-2 infection and that present in apparently normal higher primate organs.

These data, although consistent, are at present limited and the possible interpretations are, correspondingly, tentative. However, the findings are provocative and invite some speculations as to their significance. The RNAs concerned may have originated in either of two, not necessarily mutually exclusive, ways. Namely, host acquisition by an infectious process or the evolution of Ad-2 (and the C group adenoviruses) through the acquisition of nucleotide sequences similar to those found in the natural primate host.

The facts relevant to these interpretations are that only certain Ad-2 DNA regions are represented in human RNA. This tends to rule out a productive Ad-2 infection as the RNA source, as we would then expect to find RNA representative of the whole genome. Because all individuals investigated contained the RNAs in question, it is obvious that the mode of transmission is very efficient. This rules out a particular defective virus as the source unless we also assume that transmission is vertical and widespread. In this case, it is debatable whether such sequences belong to the host or to a virus. We cannot, however, exclude the possibility that the sequences we detect belong to a non-defective, incompletely Ad-2-related virus, in our view an unlikely explanation, or that Ad-2 DNA may be carried in the manner of Epstein-Barr virus²⁷, following infectious contact and subject to specific host transcriptional control.

The second hypothesis is that Ad-2 and related strains have evolved, or otherwise acquired, genes that are related to certain genes of their natural hosts. As it is known that Ad-5 can integrate with host DNA²⁸, such evolution seems likely. Also, RNA tumour viruses of chickens and mice contain sequences related to their host cells. In avian sarcoma viruses the transforming gene *src* is related in nucleotide sequence to the host gene *sarc*¹³. It is also known that the transforming region of a primate virus SV40, which is totally unrelated to Ad-2, is rather similar to Ad-2 in its transcriptional structure²⁹, although it is not known whether the proteins coded are functionally analogous. Such convergent evolution might reflect the fact that both viruses have acquired or evolved control elements concerned with the same host function, as both are primate viruses. Presumably, the advantage of such a mode of evolution would be that the virus would be better adapted to subvert host functions, for example, by using the host RNA splicing systems. Pressure to acquire control of the same system on the part of unrelated viruses could stem from the fact that the functions concerned are key elements in growth control. In this regard it is known that Ad-2 replicates in human cells significantly faster than Ad-12 (refs 30, 31), which does not, as far as we can detect, share the host-related nucleotide sequences.

Whether or not either hypothesis is wholly correct, our findings do indicate that human cells possess transcripts, from whatever source, closely related to DNA that maps within the

transforming region of the adenovirus genome. This may be relevant not only to how the virus itself replicates and transforms, but also to how malignant human cells may alter their growth patterns. Moreover, at least four regions of homology are detectable which may mean that at least four different RNAs related to the viral DNA exist in human cells. In future studies these can be recovered and their corresponding genes cloned and identified. In the event that the second hypothesis is substantially correct, such genes may relate to important functions connected with cell and virus growth control. A search for similar sequences related to other DNA viruses is also suggested from the fact that antigenic similarity has been described between SV40-induced surface antigens and fetal antigens in hamsters³².

The fact that Ad-2 early function antigens cross-react immunologically with apparently ubiquitous antigens present in the natural host, regardless of their origin, is likely to be medically significant. Bacterial pathogens that share antigens with human cells can precipitate autoimmune reactions, as in the rheumatic disease caused by *Streptococcus pyogenes* infections. Persistent adenovirus infections might therefore, by analogy, precipitate similar diseases. Thus, there might be a link between other rheumatic conditions or autoimmune diseases and the antigens that we detect. Of particular interest here is the identity of the cell type that seems to synthesise a high concentration of Ad-2-related RNA as determined by *in situ* hybridisation. If

such synthesis implies a high content of related antigens, such a cell might be particularly at risk from an autoimmune reaction directed at these common antigens.

The cellular source of the cross-reacting antigens in placental tissue is unclear. By immunofluorescence, these antigens are widely distributed in the tissue. However, they do not appear on tissue-cultured cells of human origin. Moreover, the cell type that apparently possesses the greatest amount of Ad-2-related RNA as determined from *in situ* hybridisation, and thus is presumably most likely to synthesise most antigen, is relatively rare. One possible explanation is that different RNA molecules, related to different regions of the Ad-2 genome, are not equally abundant in all cells. Another is that the antigens or their precursors circulate in blood or other body fluids and/or that their distribution is altered as a result of post-mortem changes, such as blood clotting. Such a mechanism would partly explain why these antigens have not generally been found on cells in culture.

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letters

A jet associated with M89

AN optical jet, extending 10 arc min from the elliptical galaxy M89 (NGC4552) is reported here. Two low surface brightness shells partly surround the galaxy and a much fainter arc, 13 arc min away has also been detected. The jet was found on five deep plates of the Virgo cluster obtained by the 1.2-m UK Schmidt Telescope. Three sky-limited IIIaJ (bandpass 395–530 nm) and two IIIaF (630–685 nm) plates were taken on hypersensitised¹ emulsions. A slightly different plate centre was used for two of the plates.

The faintest objects on the plates were made visible by a contact copying process designed to detect very low surface brightness structures². High contrast positives from the deepest IIIaJ plates were superimposed in register to reduce grain noise (Fig. 1). Low contrast detail, buried in the envelope of M89 was revealed by using an unsharp mask³ to remove the large-scale density variations in the images. Objects smaller than ~1 arc min were unaffected by the mask and were further

emphasised by the contrast enhancement process. Three of these high-contrast spatially-filtered positives were again superimposed in register. All the features clearly discernable on either kind of derivative made from at least two of the original plates (before superimposition) are sketched in Fig. 2, which is reproduced to the same scale as Fig. 1. A similar series of processes, using the same set of plates, was carried out on the superficially similar galaxy M84 (NGC4374). No unusual features were found.

All the derivatives from each of the plates clearly show a jet (A in Fig. 2) at a position angle of 115° extending 10 arc min (~36 kpc) from the nucleus of M89. The jet is brighter towards its tip but retains the same apparent width (~5 kpc) until it merges with the outer envelope of the main galaxy. The jet itself is slightly curved and contains some brighter condensations. One of these, (E) is off the axis of the jet and could be a background object. The outer envelope of the galaxy shows a distinct bulge on the eastern side, opposite the jet. To the south a sharply defined arc (B), reminiscent of a supernova remnant

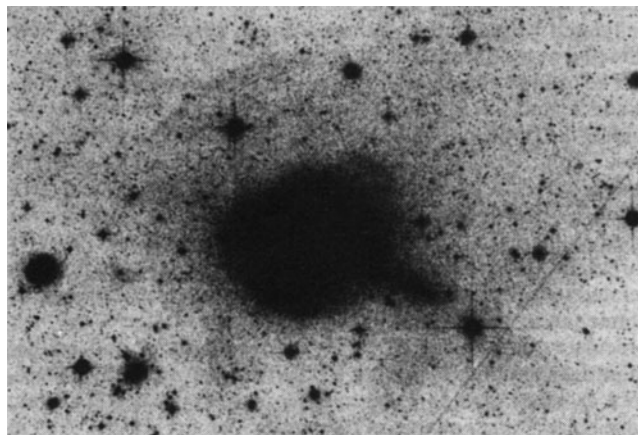


Fig. 1 Superimposed high contrast copies of two sky-limited IIIaJ plates showing the jet and three concentric shells. Same scale as Fig. 2.

shell, is visible, concentric with the nucleus of M89 and ~ 11 kpc long. The jet and southern arc are more obvious on the blue plates than on the red and are just visible on the originals. Diametrically opposite the southern bright rim and at a similar distance from the nucleus is a more diffuse arc (C) which appears as a circular patch on the red plates. The condensation D and extremely faint arc F, 47 kpc from the nucleus are evident only on the two deepest IIIaJ plates.

The dotted circle in Fig. 2 is the equivalent apparent radius of M89 at a (B) magnitude of 26.91 arc s^{-2} obtained from the photographic photometry of Fraser⁴. The circle is centred on the position of the optical nucleus.

In view of their low surface brightness, the nature of the jet and shells will be difficult to establish. Although the jet is of comparable brightness on the blue and red plates, suggesting that it is continuum emission, it could have a spectrum exhibiting [O III] 4959 and 5007 and $H\alpha$ emission lines. This would be hard to reconcile with the known lack of interstellar gas in normal elliptical galaxies. Pure continuum could indicate synchrotron radiation, but no radio jet was found in observations by Heesch⁵. The jet is therefore probably composed of stars. Tidal interaction with another galaxy cannot be discounted as the disruptive force but there seems to be no likely candidate in the vicinity and that mechanism would not account for the active radio nucleus or the concentric shells. The nucleus is a compact radio source, with a flat spectrum similar to that found in variable QSOs. The nucleus of M89 is variable at 6 cm (R. D. Ekers, unpublished data). The optical spectrum is unique among the limited number of active radio galaxies

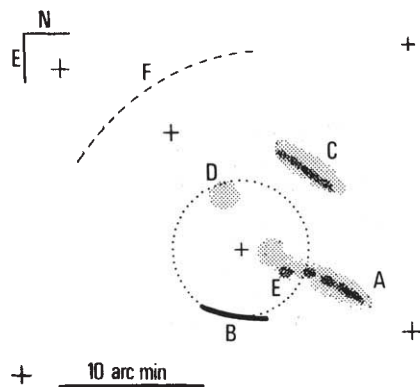


Fig. 2 Location of low contrast features visible in the spatial frequency-filtered derivatives of three IIIaJ plates.

examined by Disney and Cromwell⁶ in that it exhibits no emission lines. It seems likely that the jet and concentric shells are stars displaced by one or more violent outbursts in the active nucleus of the galaxy.

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Theory of the Earth-synchronous rotation of Venus

CARPENTER¹ noted in 1966 that the radar observations of the rotation period of Venus gave a figure remarkably close to one at which Venus would present the same aspect to the Earth at each inferior conjunction. Goldreich and Peale² discussed this as a possible resonance with a gravitational couple exerted by the Earth on a permanent transverse quadrupole moment of Venus, but noted that such a couple would be expected to be much smaller than that due to the Sun acting on the solid body tide. The rotation would continue to slow down due to tidal friction, and could not have been arrested in the resonance. We therefore proposed³ that the solar couple on thermal atmospheric tides might be expected to apply an acceleration to the spin of Venus, thus opposing the body tidal frictional deceleration. (An effect in that sense occurs for the Earth.) The two opposed torques would have a different dependence on the rotation speed of Venus, and might balance at a particular value. As the rotation speed approached this value, the torque necessary for capture into a resonance would have become very small, and the effect exerted by the Earth might then be sufficient. A diurnal pressure wave of as little as 1 mbar could balance the body tide. Ingersoll and Dobrovolskis⁴ have reconsidered this suggestion, and attempted a quantitative calculation of the atmospheric tide. Although their result seems to favour the theory, we show here that their approach is not valid. Ingersoll and Dobrovolskis consider a quasi-static atmosphere heated diurnally, and then calculate the mass distribution and hence the solar couple that results. But the quasi-static assumption is not justified for the known circumstances on Venus. Wind speeds must be expected⁵, and have indeed been observed⁶, that cause an advection of heat substantially faster than the motion of the subsolar point over the surface ($\sim 4 \text{ m s}^{-1}$); in these circumstances the atmospheric mass distribution will be determined by the dynamics of the convecting atmosphere. (The acceleration terms rather than temperature make the main contribution to horizontal variations of pressure and density.) A secure result cannot be obtained until the flow patterns and velocities are known, from a detailed theory of planetary convection, or from direct observation.

Some considerations can immediately be used to show that the dynamical effects are likely to be as strong or stronger than we showed to be needed for the resonance capture. First, wind speeds on Venus are fast relative to the planet's rotation rate, unlike the case for the Earth. The slow diurnal rotation (117 d) and the deep, radiation-absorbing atmosphere of Venus make it probable that most of the global convection pattern is relatively stationary with respect to the subsolar point, rather than being carried around with the planetary rotation as is the case for the Earth. Cloud pattern photography, for example, of the permanent disturbance downwind from the subsolar point,

substantiates this, but is available as yet only for the upper atmosphere⁷. The atmospheric mass asymmetries created by this motion are subject to a continuous solar torque. Indeed they may help produce the high altitude 4-d circulation⁸.

Second, the wind speeds of such an advection have been estimated by several authors⁹, and speeds of the order of 5 m s^{-1} are expected in the densest levels of the atmosphere (above the surface boundary layer). On a slowly rotating planet, the dynamical pressure fluctuations associated with this, such as the pressure drop in a large eddy, are then of the order of ρv^2 , where ρ is the density and v the wind speed. This suggests that variations of 15 mbar in the surface pressure are to be expected, a figure substantially larger than the minimum of 1 mbar that we showed to be adequate for the tidal stability of the resonant rotation.

Soviet measurements⁶ with Veneras 9 and 10 found wind speeds of $40\text{--}50 \text{ m s}^{-1}$ down to 30 km above the surface. The corresponding value for ρv^2 is $\sim 180 \text{ mbar}$, and similar absolute values of the pressure fluctuations are then expected to occur down to the surface. These measurements, which generally confirm the calculations of Gierasch *et al.*⁹, also indicated that wind speeds were similar over a substantial ($\sim 20^\circ$) range of longitudes¹⁰, making it clear that they were associated with large-scale patterns rather than merely small-scale turbulence that would be inconsequential for tidal effects. The figure of 180 mbar is so much in excess of what is required that the distribution of the atmospheric mass asymmetry can be very inefficiently organised and still be enough to stabilise the resonant rotation against solid body tidal drag.

We conclude that the dynamical effects of convection will give the Venus atmosphere asymmetries in its mass distribution with respect to the Sun that are fully expected to provide an adequate couple for the Earth-resonance theory. A more detailed discussion depends on an improved understanding of the entire atmospheric dynamics.

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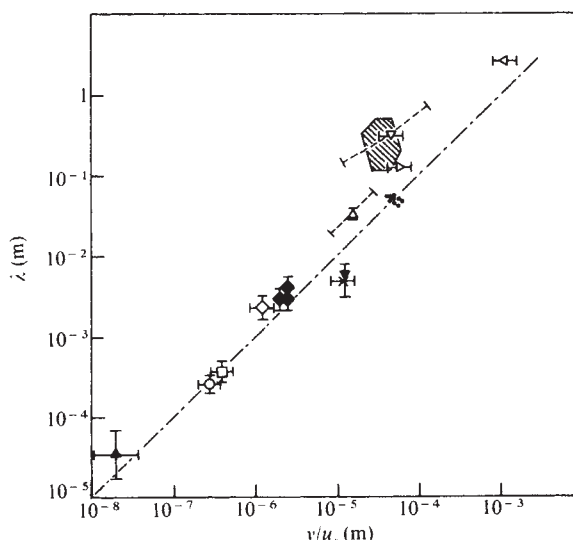
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Size of scallops and ripples formed by flowing water

THE passage of water over a soluble solid wall often gives rise to intersecting polygonal depressions or horseshoe-shaped pits known as scallops and flutes^{1,2}. The geometry of these surface deformations is remarkably reproducible in given flow conditions, but there is no generally agreed theoretical framework for predicting their characteristic dimensions, such as the spacing between successive transverse ridges. I present here indirect evidence in support of the hypothesis that the scalloping of soluble surfaces is due to the imprint of eddy patterns inherent in turbulent flow near a passive wall, and argue that small ripples on a granular bed may be formed in essentially the same way.

The attribution of bedform morphology to the structure of wall turbulence, rather than to the physical properties of the bed itself, has been suggested several times^{3–6}. We may call this basic assumption the wall-similarity hypothesis, since it implies by dimensional arguments referred to in the turbulence literature as the principle of wall similarity⁷, that a characteristic length scale of the bedform should be a universal multiple of ν/u_* , where ν is the kinematic viscosity and u_* is the friction velocity. Although the wall-similarity hypothesis has been shown to account successfully for the size of scallops formed by dissolution both in geophysical flows⁵ and in laboratory studies⁶, its validity range is uncertain¹, and in particular no attempt has been made to apply it to features observed on granular beds. Among the diverse bedforms associated with non-cohesive sand, those described as ripples⁸ resemble dissolution scallops most closely in size, and hence have the strongest claim to an explanation in terms of the wall-similarity hypothesis. However, it has been demonstrated experimentally^{9,10} that ripple wavelength does not depend in a simple linear manner on ν/u_* , and this has been taken to mean that wall-similarity concepts are not relevant to sand beds¹¹.

Most of the literature on bedform morphology refers to flows occurring in nature or to laboratory simulations of geophysical flows, but several instances have been reported of flow-induced wall deformations in man-made industrial plant. A striking example is the formation (probably by a dissolution process) of



key	fluid	wall	remarks	ref.
■	cold & warm water	granular, non-cohesive	laboratory flume	9
*	"	"	"	own expts.
○	hot water	magnetite	boiler tubes	12–18
△	cold & warm water	plaster	laboratory channel	6
▷	cold water	rock	natural formations	5
◁	air	ice		
▼	cold water	colloidal particles	deposits in a water main	24, 25
▽	"	ice	ripples on the underside of river ice	26
◆	warm water	bitumen	laboratory pipe-flow expts.	22
□	cold & warm water	copper	laboratory studies of erosion corrosion in pipes	21
◇	cold water	brass	"	20
▲	water at 750 m s ⁻¹	aluminium	laboratory studies of erosion by droplets	23
×	cold water	machined ripples	see text	37

Fig. 1 Streamwise spacing of flow-induced surface deformations, λ , plotted against the wall-similarity parameter, ν/u_* . Chain-dotted line: $\lambda = 10^3 \nu/u_*$. Broken lines inclined to the axes indicate the range of conditions covered by the original references.

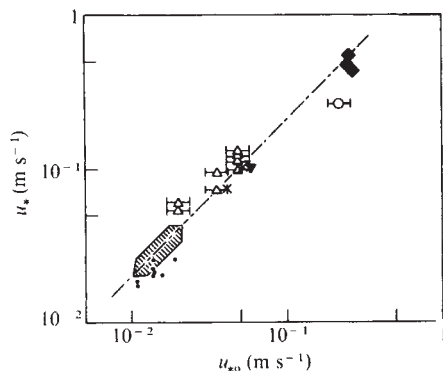


Fig. 2 Empirically determined friction velocity, u_* , for flow over naturally scalloped or rippled surfaces, plotted against the friction velocity, u_{*0} , calculated or measured for the same bulk flowrate over a smooth wall. For the key see Fig. 1. Chain-dotted line: $u_* = 2u_{*0}$.

rippled layers of magnetite on the internal surfaces of boiler tubes carrying hot water at a velocity of a few metres per second¹²⁻¹⁸. The morphology of the deposits is very like that of cave scallops or river-bed ripples, but the longitudinal wavelength is much smaller, of the order of 200–350 μm . Such a reduction of scale agrees well with wall-similarity predictions.

Several published accounts of wall deformations generated by flow are summarised in Fig. 1. The reported morphologies differ in detail, but all the deformations can be described as either scallops or ripples, displaying a definite transverse character and a well-defined average spacing, λ , in the direction of flow. Some of the original papers do not give sufficient information to permit exact calculation of ν/u_* . In these cases, plausible assumptions have been made regarding water temperature or Reynolds number, and where necessary u_* has been taken to be twice the friction velocity appropriate to a smooth wall at the same bulk flowrate, as calculated using the Moody chart¹⁹ for pipe flows or by assuming a universal logarithmic velocity profile⁷ for the flume and channel flows. This method of estimating u_* was based on an analysis of those sources giving information on the increase in resistance to flow accompanying the formation of scallops and ripples (Fig. 2).

The approximate proportionality apparent in Fig. 1 between λ and ν/u_* over a range exceeding four decades of length represents strong support for the wall-similarity hypothesis. Most of the data are fitted within a factor of two or three by the formula $\lambda = 10^3 \nu/u_*$. Figure 1 includes some of my experimental points, obtained using a variety of granular beds, of order 1 mm thick, in a small laboratory flume. My results, considered in isolation, do not show the trend $\lambda \propto \nu/u_*$, but the group of points taken as a whole falls on the same line as data referring to the corrosive dissolution of steel¹²⁻¹⁸, brass²⁰ and copper²¹, the plastic shear of bitumen²² and aluminium²³ and the rippling of colloidal-particle deposits in a water main^{24,25}. On deeper sand beds⁹, ripple wavelengths are larger by a factor of ~ 4 than those obtained in my tests, suggesting that the height attained by ripples, ϵ , may influence their spacing; this idea is supported by the fact that as ripples develop from an initially plane bed, their wavelength increases until eventually stabilising as an equilibrium ripple height is reached²⁷. Linear regression applied to the deep-bed data in the form $\lambda = A\nu/u_* + B\epsilon$ yielded $A = 1,250$, in satisfactory agreement with my shallow-bed results, and $B = 13.3$, a value consistent with the commonplace visual observation that in the lee of a ripple crest there is an unsteady recirculation zone extending a distance of the order of 10ϵ downstream. However, allowance for ripple height does not adequately account for the anomalously large spacing of river-ice ripples²⁶, and it is possible that additional corrections to the basic wall-similarity estimate, as yet unidentified, will prove necessary for the accurate prediction of λ .

As there is no systematic divergence in Fig. 1 between data points relating to the rippling of granular beds or plastically deformable surfaces and points referring to dissolution processes, it seems that the applicability of the wall-similarity hypothesis is not limited to dissolution scallops alone. The evidence suggests the size of both scallops and ripples is determined primarily by the scale of pre-existing eddies in the adjacent turbulent flow. If circumstances permit the surface deformations to become so pronounced that extensive regions of separated flow are set up behind crests, the morphology and characteristic dimensions of the deformations will in general be modified, but it seems that this should properly be regarded as a secondary complication. Flow separation may perhaps be best considered as responsible merely for furnishing final sculptural details, determining, for example, whether scallops are formed rather than ripples, while the overall scale of the bedform has its origin in eddy patterns already present in the flow before surface irregularities have become sufficiently marked to induce separation. (These remarks do not, of course, apply to large-scale bedforms such as dunes or antidunes.)

If this perspective is accepted, the fact that the wavelength of ripples on a granular bed does not vary directly with ν/u_* is no longer irreconcilable with wall-similarity ideas. Suppose, for example, that the amount by which u_* exceeds the threshold of movement is a factor affecting bedform length scale, possibly through its influence on ripple profile and hence on the extent of the separation zone in the lee of a crest. Such a second-order effect, although subsidiary in the sense that it operates in the context of a bedform whose characteristic dimensions are fixed principally by turbulence length scales may, nevertheless, mask the simple linear dependence of λ on ν/u_* when the latter quantity is varied over the necessarily restricted range available in flume experiments, where u_* is bounded below by the threshold of movement and above by the necessity of preventing the ripples giving way to a different bedform regime.

The wall-similarity hypothesis for the rippling of granular beds may also provide an explanation for the absence of ripples when grains are larger than a critical size. For grain Reynolds numbers which are not too small, the Shields criterion is⁸ $u_{*c} = 0.2(\Delta\rho g d/\rho)^{1/2}$ where u_{*c} is the friction velocity at the threshold of movement, ρ is the water density, $\Delta\rho$ is the difference in density between particle and fluid, d is the particle diameter and g is the gravitational acceleration. The maximum observable ripple wavelength for a bed of grain size d is therefore

$$\lambda_{\max} = 1,000 \nu/u_{*c} = 5,000 \nu(\Delta\rho g d/\rho)^{-1/2}$$

But as the height of a fully developed ripple is usually no more than a tenth of its wavelength, a ripple will be indistinguishable from background bed roughness unless $\lambda_{\max} > 20d$, say. Hence the grain size above which rippling would not be observed may be estimated as

$$d_{\max} = (250\nu)^{2/3} (\Delta\rho g/\rho)^{-1/3}$$

Inserting physical properties appropriate to sand and water at ambient temperatures, the predicted $d_{\max}$ is 1.6 mm, which compares favourably with the value of about 0.7 mm found empirically¹¹.

Although no mechanistic explanation can be given for the value of the constant of proportionality between λ and ν/u_* , its order of magnitude suggests that the eddies responsible for the formation of scallops and ripples may be identified with the coherent flow structures known as sublayer bursts^{28,29}. The dynamics of sublayer bursts are not understood, but empirical studies of turbulent flow over a smooth wall have established that they are elongated in the streamwise direction, with a typical lateral spacing³⁰⁻³³ of $100 \nu/u_*$ and a longitudinal length scale²⁹ in the approximate range $1,000$ – $1,500 \nu/u_*$. Relatively few experiments have been performed to examine how the behaviour of sublayer bursts is modified by surface roughness^{34,35}, but in the case of a wall roughened by transverse ribs the streaky structures associated with bursts have been

observed in the regions between ribs³⁴. The bursts, which near a smooth surface occur randomly in position and time, possibly become locked into the irregularities of a deforming wall, thereby imposing bedform dimensions which reflect their own characteristic longitudinal scale, approximately $10^3 \nu/u_*$. This speculation receives some support from evidence that bursts can impose their lateral length scale when longitudinal grooves are formed on a soluble² or erodible^{35,36} wall. It is also interesting that as the flowrate is varied over a wall into which artificial ripples have been machined³⁷, the resistance to flow passes through a maximum when the ratio between ripple spacing and ν/u_* is $\sim 10^3$ (Fig. 1). Such a resonance phenomenon is consistent with the postulated tendency of sublayer bursts to lock into a spatially periodic pattern of transverse ridges: at resonance the surface morphology can be envisaged as giving the maximum possible assistance to the bursts in projecting low-momentum fluid towards the core of the flow, augmenting the Reynolds stress.

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Iron-nickel 50–50 superstructure in the Santa Catharina meteorite

MÖSSBAUER effect and X-ray diffraction measurements have shown that thin lamellae of the Cape York and Toluca meteorites contain the ordered phase Fe–Ni (50–50%) with the structure L10 (refs 1–3). The bulk of these meteorites consists mainly of body centred cubic (b.c.c.) iron–nickel α -phase alloy. We report here Mössbauer spectra, electron microprobes and X-ray measurements which show that in contrast to this, the

bulk of the Ni-rich ($\approx 35\%$) Santa Catharina Ataxite consists of up to 50% Fe–Ni superstructure⁴.

Thin slices of the bulk of the Santa Catharina meteorite from specimens of the collection from the Museu Nacional in Rio de Janeiro were cut with a precision saw, carefully avoiding thermal stresses in the sample. Absorbers for Mössbauer spectra measurements were prepared by polishing the slices to a thickness around 70 μm . The spectra were recorded with a 20 mC source ^{57}Co in Rh matrix with a velocity drive coupled to a multichannel analyser of 1,024 channels. A 370/145 IBM computer was used for folding and analysing the data with a lorentzian least square fitting.

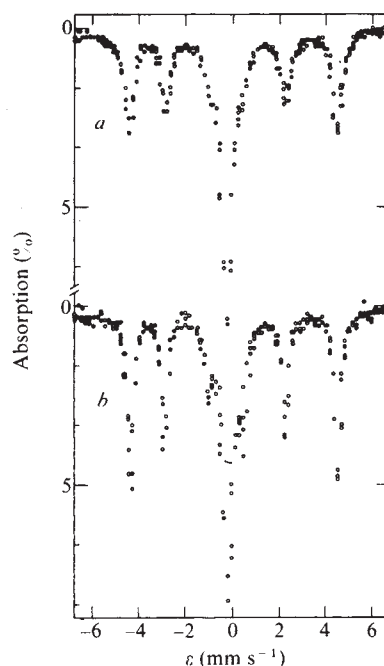


Fig. 1 Mössbauer absorption spectra of: a, a thin slice ($\approx 70 \mu\text{m}$) of the Santa Catharina meteorite; and b, thin lamellae of the Toluca meteorite. The spectra were recorded at room temperature ($\approx 22^\circ\text{C}$). Source, ^{57}Co in Rh.

Figure 1 compares a spectrum of the Santa Catharina sample with one obtained in the same conditions with a thin lamella of the Toluca meteorite, in which X-ray determinations showed the presence of the superstructure³. Both spectra are a superposition of two spectra: (1) a central line from a paramagnetic phase; and (2) an asymmetric 6-line spectrum, due to the L10 superstructure, which gives rise to a quadrupole splitting caused by the non-cubic surroundings of the iron atoms in this alloy^{5–7}. The magnetic hyperfine field H_i and the electric quadrupole shift ϵ of the Santa Catharina sample are about the same as those observed with the lamella (lamella: $H_i = 289 \pm 2 \text{ kG}$, $\epsilon = 0.20 \pm 0.02 \text{ mm s}^{-1}$; Santa Catharina: $H_i = 291 \pm 3 \text{ kG}$, $\epsilon = 0.17 \pm 0.02 \text{ mm s}^{-1}$). The linewidths obtained with the Santa Catharina ($\Gamma \approx 0.52 \text{ mm s}^{-1}$) are larger than those obtained with the Toluca lamella ($\Gamma \approx 0.32 \text{ mm s}^{-1}$). This may be due to the larger thickness of the sample. Only minor changes, both in the paramagnetic central line and in the magnetically split Mössbauer spectrum, were observed on going from room temperature to liquid nitrogen (77 K) and liquid helium (4.2 K) temperatures. This surprising result, will be discussed in detail elsewhere.

As has been observed with the Cape York lamellae², the Mössbauer spectra of samples from the Santa Catharina meteorite at 732 K show that the breakdown of the superstructure occurs over a time scale of $\sim 10 \text{ h}$.

From our results, the area ratio of the 6-line spectrum to the single line, we conclude that ~50% of the Santa Catharina meteorite consists of the ordered Fe-Ni alloy with L10 structure. In the limits of sensitivity of the Mössbauer effect no evidence was found for other iron-containing minerals.

Electron microprobe (Microscan 5, Cambridge Scientific Instruments) analysis of the polished Santa Catharina samples

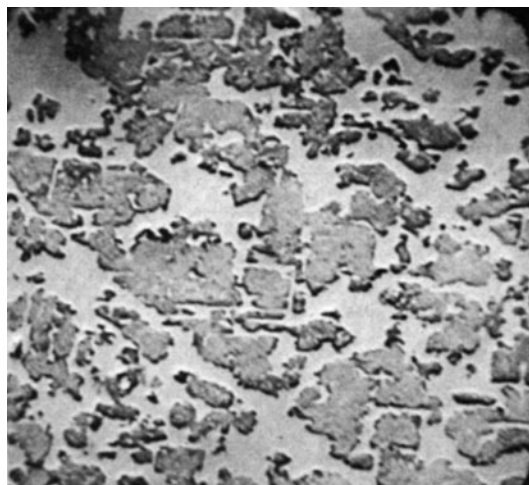


Fig. 2 Back-scatter electron image from electron microprobe analysis of a polished surface of the Santa Catharina sample. From point analysis determination it is found that the dark areas correspond to the Fe-Ni ordered phase whereas the light ones correspond to the Fe-rich paramagnetic phase.

indicates clearly the presence of at least two phases⁸ as shown in Fig. 2. Microanalysis at points of the two phases indicate, from an average of 50 determinations, that the darker phase has a homogeneous composition, 51–50% (by weight) of Ni and 49–48% of Fe and that the lighter phase has a more heterogeneous composition containing nearly 27% of Ni. The meteorite consists of an intergrowth of orientated domains of these two phases, which implies that slices cut from the meteorite will always contain both phases. The presence of oxygen and sulphur in maximum amounts of 1–2% were found uniquely on the Ni-rich phase. Larger proportions of oxygen have been reported in this phase from earlier microprobe work⁹. The discrepancy may be due to the fact that earlier measurements were made on a severely oxidised sample. The fact that our samples taken from the bulk of the Santa Catharina meteorite show only traces of corrosion demonstrates that care has to be taken when results from different specimens of the Santa Catharina meteorite are compared¹⁰. We have also studied the sample from Santa Catharina by X-ray techniques. Oscillating crystal photographs show that the sample over areas of at least 1 mm² is essentially a single crystal, and that it contains the superstructure L10 of Fe-Ni. The X-ray photographs showed no traces of martensitic α_2 phase.

Remarkably ~50% of the Santa Catharina meteorite, which originally may have been a 25,000 kg meteorite¹¹, consists of the ordered Fe-Ni structure. This structure has only been obtained by strong neutron or electron irradiation of disordered alloys at temperatures near the ordering temperature^{5–7} ($T_c \approx 320^\circ\text{C}$). Diffusion processes substantially below T_c are too slow to obtain thermodynamic equilibrium¹². The presence of the ordered Fe-Ni in Santa Catharina and other meteorites^{1–3} is probably due to the slow cooling of the meteorite parent body.

Further experiments are in progress to clarify the nature of the iron-rich paramagnetic phase as well as the magnetic properties of the ordered Fe-Ni phase.

Microprobe analysis were performed by H. Schorscher, C. Wiedmann, and C. Jehanno.

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Paraffinic hydrocarbons from supercritical-gas extracts of coal as organic geochemical markers

PARAFFINIC hydrocarbons have been proposed as indicators of the conditions obtaining during the deposition and subsequent maturation of sediments^{1–4}. We consider here the relationship between the rank (as measured by carbon content) of coals and analytical results for the alkane fractions obtained by a novel extraction procedure (under investigation for the derivation of liquid fuels and feedstocks from coal^{5,6}) which uses super-critical gases (SCG). We first discuss the limitations of *n*-alkane distributions and then deal with acyclic isoprenoids, drawing particular attention to the ratio of pristane to phytane.

In the *n*-alkane fractions of coals, like those from some other sediments, odd carbon numbers predominate over even³. For a series of Saar coals the carbon-preference index (CPI) varied systematically from 1.59 to 1.00 with degree of coalification⁷, and a related parameter dropped⁸ from 15.3 to ~1.0 for Australian coals as the carbon content changed from 67% to over 90%. On the other hand, the CPI of *n*-alkanes from hatchettites (coal-associated minerals) varied only between 0.96 and 1.06 (ref. 9). Recently, White *et al.* have questioned the correlation between *n*-alkane distributions and coal rank for paraffins from coal hydrogenated in the SYNTHOIL process¹⁰.

We have isolated paraffinic hydrocarbons from a variety of coal derivatives by silica-gel chromatography of the portion soluble in *n*-pentane. Gas chromatographic analyses were then made on both packed and capillary columns for whole alkane fractions and for the straight-chain and branched-plus-cyclic alkane fractions separated on a molecular sieve^{11–13}; mass spectrometry enabled unambiguous identification of individual alkanes¹². Table 1 and Fig. 1 present our results for the distributions of *n*-alkanes derived from SCG and Soxhlet extraction, and low-temperature carbonisation from UK, Turkish and

Table 1 Origin of coal extracts and properties of derived aliphatic hydrocarbon fractions

Coal source	% Carbon of coal (dry ash-free basis)	Origin of extract	Aliphatic fraction % of coal	<i>n</i> -Alkanes % of coal§	CPI* of <i>n</i> -alkanes	Significant† concentration of alkenes	Isoprenoids p.p.m. of coal§	Pr/Ph	Ref.
Turkish lignite 1 (Elbistan)	67.3	SCG extraction at 340 °C and 8 MPa‡	0.7	0.45	1.47	No	150	1.6	This work
		SCG extraction at 400 °C and 17 MPa	2.0	0.8	1.16	Yes	ND	ND	This work
Turkish lignite 2 (Seyitomer)	71.1	SCG extraction at 340 °C and 8 MPa	0.7	0.5	1.13	No	120	2.0	This work
Turkish lignite (Tunçbilek)	74.6	SCG extraction at 400 °C and 17 MPa	ND	ND	0.99	Yes	ND	1.0	This work
Sigma, South Africa	76.7	SCG extraction at 350 °C and 17 MPa	0.09	0.06	1.33	Not stated	NA	NA	14
		SCG extraction at 450 °C and 17 MPa	0.5	0.3	1.01		NA	NA	14
		Soxhlet extraction with 3:1 chloroform/methanol for 100 h	0.05	0.03	1.93		NA	NA	14
Pittsburgh, US	80.6	Low-temperature carbonisation (COED process) ²⁵	NA	NA	1.02	Yes	NA	6.0	This work
Thoresby, UK (CRC 801)	81.4	Low-temperature carbonisation (Rexco process) ²⁶	~0.5¶	~0.3¶	1.02	Yes	~200¶	4.3	This work
Daw Mill, UK (CRC 902)	82.6	SCG extraction at 400 °C and 10 MPa	0.6	0.3	1.04	Yes	200	2.9	This work
		Soxhlet extraction with 7:3 benzene/ethanol for 50 h	0.07	0.03	1.07	No	70	2.6	This work
Markham Main, UK (CRC 802)	82.7	SCG extraction at 350 °C and 10 MPa	0.35	0.2	1.09	No	500	5.6	11
		SCG extraction at 400 °C and 10 MPa in presence of H ₂ /ZnCl ₂	1.6	0.5	1.08	No	500	3.5	This work
		Low-temperature carbonisation at 430 °C (Roomheat process)	~0.4¶	~0.3¶	1.02	Yes	~150¶	4.4	This work and 15
		Soxhlet extraction with 7:3 benzene/ethanol for 50 h	0.08	0.04	1.27	No	110	4.2	This work

NA, not available; ND, not determined.

* CPI = $\frac{1}{2} \{ \text{odd } (C_{15}-C_{29}) / \text{even } (C_{14}-C_{28}) + \text{odd } (C_{15}-C_{29}) / \text{even } (C_{16}-C_{30}) \}$

† >0.01% of extract regarded as significant.

‡ Toluene, used as solvent for all SCG extractions, yielded insufficient Soxhlet extract for alkane analysis.

§ GC conditions: 50 m × 0.3 mm glass capillary column coated with OV-101 programmed from 373 to 473 K at 1.5 K min⁻¹, and for higher paraffins, a 2 m OV-1 column.¶ Based on 10% tar yield²⁶.

US coals, together with Kershaw's data for extracts of a South African coal¹⁴. By volatilising liquids otherwise insufficiently volatile to distil at the temperatures used, the SCG extraction process^{5,6} yields much greater amounts of paraffins than conventional extraction procedures, but without appreciable hydrogen transfer¹¹.

The low C₁₀–C₁₅ *n*-alkane contents in Fig. 1a, c, d, and g provide some evidence that the alkane fractions are not significantly degraded during SCG extraction over brief periods at around 340 °C. Indeed, although SCG extraction at 350 °C of the Markham Main (UK) and Sigma (South African) coals produces much greater quantities of *n*-alkanes than the mild Soxhlet extraction process (Table 1), the *n*-alkane range is almost identical (compare Fig. 1d with f, and g with i). The virtual absence (<0.01%) of alkenes in low-temperature SCG extracts (Table 1) also suggests a very low degree of pyrolysis. On the other hand, alkanes from a tar generated by fluidised-bed carbonisation¹⁵ of Markham Main coal showed a roughly gaussian distribution (Fig. 1h), with a shift to lower carbon number compared with SCG extraction (Fig. 1g); significant quantities of alkenes detected in this and other low-temperature tars (Table 1) are thought to arise by de-methanation and de-ethanation of *n*-alkanes. Moreover, even SCG extraction at only 400 °C generated alkenes from Turkish lignites, and shifted the *n*-alkane distribution from that of a 340 °C extract (compare 1a with 1b); the CPI fell from 1.47 to 1.16 (Table 1). Alkenes were also apparently present in the 400 °C SCG extract of Daw Mill coal¹³. CPI values for the *n*-alkanes derived from Kershaw's data¹⁴ also illustrate the effect of increasing the SCG extraction temperature from 350 °C (CPI 1.33) to 450 °C (CPI 1.01). Other SCG extracts at 400 °C, as well as low-temperature tars, exhibit CPI values near unity (Table 1).

Because it yields much larger quantities (by up to two orders of magnitude) of paraffins, SCG extraction without pronounced cracking, should provide alkanes that are more representative of

the whole coal than does simple extraction (which yields only 0.007–0.099%, in refs 8, 10 and 14 and this work). Now, two sources of alkanes in coals must be considered: (1) the material physically absorbed in the micropore structure¹⁶; and (2) the compounds generated by chemical breakdown of oxygen-containing compounds, or long alkyl chains, shown¹³ by ¹³C NMR to be bonded within the coal matrix.

Vahrman attributed the increasingly smooth carbon-number distribution of *n*-alkanes from coal as extraction times become more extended to the differences in accessibility of the *n*-alkanes with high CPI (from the leaves, cuticle, and so on of the plants from which the coal is derived) as compared with those of lower CPI (originating from heartwood)¹⁶. A similar effect is evident when one compares CPI 1.27 for Soxhlet extraction (benzene-ethanol gave better yields than toluene) with CPI 1.09 for SCG extraction at 350 °C of the Markham Main (UK) coal; for the South African coal, the CPI decreases from 1.93 to 1.33 for analogous extracts. However, Allan *et al.* have shown that moderate temperature increases (from 275 to 375 °C for 24 h) can substantially change *n*-alkane distributions from UK vitrinites¹⁷. Evidently the extra alkanes generated on heating vitrinites have a different degree of odd/even predominance from those accessible to Soxhlet extraction; thus one cannot distinguish between the two processes, (1) and (2) above, as to the source of the extra *n*-alkanes in the SCG extracts. On the other hand, coal hydrogenation procedures, such as the SYNTHOIL process¹⁰ and hydrogen-assisted SCG extraction (Table 1), clearly yield even larger quantities of *n*-alkanes, probably by hydro-cracking of aliphatic side chains.

Thus, apparent correlations between CPI and coal rank are almost certainly spurious because they are based on atypical proportions of *n*-alkanes obtained by simple extraction; furthermore, CPI values for *n*-alkanes derived by processes involving heating above 350 °C, or by hydrogenation, are also unreliable. SCG extraction at 340–350 °C may yield more

representative samples, but the variety of origins of the alkanes extracted in this way renders the CPI of doubtful value in correlations with coal rank: CPI values for four coals from diverse sources show only a very approximate correlation with dry-ash-free carbon content (Table 1). All that can be said is that the large concentrations of C_{23} and higher n -alkanes are consis-

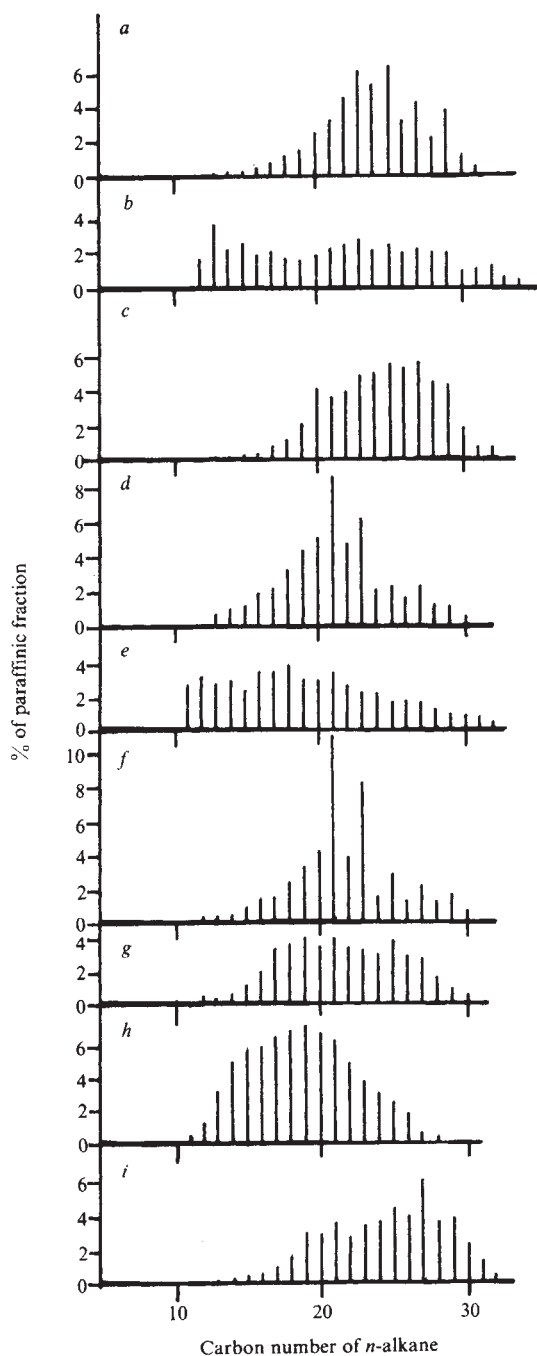


Fig. 1 Distributions of n -alkanes in paraffinic fractions of coals: *a*, Turkish lignite 1 (Elbistan), SCG extraction²⁷ at 340 °C; *b*, Turkish lignite 1, SCG extraction at 400 °C; *c*, Turkish lignite 2 (Seyitomer), SCG extraction²⁷ at 340 °C; *d*, Sigma (South African) coal, SCG extraction at 350 °C; *e*, Sigma (South African) coal, SCG extraction at 450 °C; *f*, Sigma (South African) coal, Soxhlet extraction with 3:1 chloroform/methanol for 100 h; *g*, Markham Main (UK) coal, SCG extraction at 350 °C; *h*, Markham Main (UK) coal, fluidised-bed carbonisation at 430 °C; *i*, Markham Main (UK) coal, Soxhlet extraction with 7:3 benzene/ethanol for 50 h. (*d*), (*e*) and (*f*) from ref. 14.

tent with the predominantly land-derived nature of each sediment; a marine origin is typified by lower chain lengths^{1,2} (Fig. 1).

Acyclic isoprenoids may provide clearer evidence than n -alkanes of the geochemical history of sediments² as their major precursor is thought to be the phytol side chain of the chlorophyll pigment from the original plant material. The presence of six derived hydrocarbons (C_{14} , C_{15} , C_{16} , C_{18} , C_{19} and C_{20} isoprenoids) has been demonstrated in numerous coal extracts and tars³. In Australian sub-bituminous coals¹⁸, the pristane (C_{19}) content of the alkane fraction of (low-percentage) chloroform-methanol extracts begins to increase as carbon contents exceed 76%, but the phytane (C_{20}) concentration does not increase significantly until much later in the diagenetic process, corresponding to 83–85% carbon. At this stage the pristane/phytane ratio (Pr/Ph) is a maximum¹⁸. Parallel relations have been demonstrated between Pr/Ph and reflectance of New Zealand vitrinites¹⁹, and with the maturity of shales from the Douala Basin²⁰. The inter-relation of coal maturation and oil formation^{18,21} is emphasised by correspondence between Pr/Ph for oils and the rank of coals in associated beds²². These changes during maturation, however, may not entirely erase the partial record of the palaeoenvironmental conditions during sedimentation provided by Pr/Ph²; by increasing the relative proportion of pristane to phytane, thermal diagenesis preserves Pr/Ph ratios greater than one, typical of deposition in oxic conditions^{2,22}.

In contrast with the CPI of n -alkanes, and in spite of the threefold increase in the quantity of isoprenoids liberated during SCG extraction, Pr/Ph ratios are similar for SCG and Soxhlet extracts and, even for low-temperature tars, from the same coal (Table 1). Moreover, even though the coals are of diverse origin, the Pr/Ph ratios still fit fairly closely to the curve for simple extracts determined for Australian coals by Brooks *et al.*¹⁸. The consistency of the Pr/Ph ratio in differing extraction conditions, and the similarity in thermal stabilities of isoprenoids reflected in the agreement for low-temperature tars, clearly make this parameter useful in discussing the geochemical history of coals, although more than one origin is possible for these compounds.

The isoprenoids form a higher proportion of the total paraffins from Soxhlet extracts than from SCG extracts, in keeping with Vahrman's suggestion¹⁶ that branched alkanes are more accessible within the micropore structure. However, as for n -alkanes, the release of isoprenoids bonded within the coal structure must also be taken into account; Connan has shown¹⁹ how larger quantities of pristane and phytane were extractable from lignite after heating for 3 days at 300 °C.

The completeness of extraction of isoprenoids by the SCG method is indicated by the similarity of the total contents (expressed as a fraction of the original coal) in products obtained at 350 °C and in the presence of hydrogen at 400 °C (Table 1). Indeed, for the Markham Main coal, isoprenoid content (with pristane predominant at as much as 300 p.p.m.) suggests that much of the chlorophyll-derived phytol remains in 'fossil' form. The biochemistry of Palaeozoic flora is obscure; modern descendants of such plants may differ markedly in their environments and occupy different ecological niches²³. However, if a chlorophyll content²⁴ of 0.7–1.3% (dry basis) for higher plants including ferns and mosses is taken as appropriate, the total available phytol would then amount to between 2,000 and 4,000 p.p.m. of this, compared with 500 p.p.m. derived isoprenoids in the coal.

In conclusion, SCG extraction at about 350 °C causes little thermolysis and is favourable for extracting relatively large quantities of paraffins representative of the original coals. Because of the different nature of n -alkanes physically absorbed in the coal, and at least partly accessible to Soxhlet extraction, as compared with those chemically released by heating, the CPI is inappropriate for monitoring deposition and maturation. The isoprenoidal hydrocarbons, and especially the Pr/Ph ratio, are more widely applicable as geochemical markers, and valuable

for commercial products derived from coal by newer liquefaction processes.

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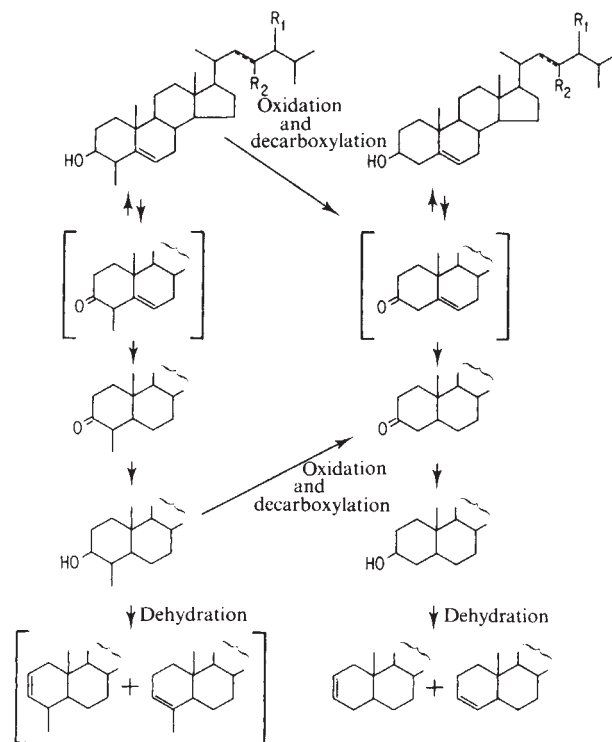


Fig. 1 A proposed mechanistic pathway for the microbiological or chemically mediated degradation in sediments of sterols to sterenes through stanone intermediates. Compounds in parenthesis are intermediates not isolated in this study.

the diagenesis of sterols to sterenes, a group of unsaturated steroids detected in some marine sediments¹ (Fig. 1). We report here the isolation, identification, and geochemical significance of a series of 4-methyl stanones and their 4-desmethyl counterparts from anoxic surface sediments in an upwelling zone on the south-west African shelf, Walvis Bay, RSA. To our knowledge, this is the first report of stanones being observed in Recent marine sediments, although 4 α -methyl stanones have been reported previously as major components of the immature Messel oil shale².

Sediment samples were collected on RV Atlantis II Cruise 93, Leg 3, Capetown to Capetown, RSA in December 1975–January 1976. Core 16, taken from Station 11 at a water depth of 49 m at 22°47.5'S, 14°25.8'E, was analysed for its sterol (sterol + stanol), stanone, and sterene contents. The geology of this area has been described by Bremner^{4,5} and the advantages of doing organic geochemical research in Walvis Bay by Boon *et al.*^{6,7} and Morris and Calvert⁸. The sediment analysed was an organic-rich diatomaceous mud, composed mainly of frustules of *Coscinodiscus*, *Actinocyclus*, and *Thalassionema*. The top 4 cm of sediment were treated with 3 M HCl to remove the large amount of carbonate and Soxhlet extracted with benzene-methanol as previously described³. Column chromatography of this extract was performed on a 5% deactivated silica gel column using increasing amounts of ethyl acetate in hexane as eluant³. Fraction 1, eluted with hexane, contained various alkanes, cycloalkanes, alkenes, cycloalkenes, and sterenes. Fraction 6, eluted with 15% ethyl acetate in hexane, contained the steroid ketones. Androstane was added as an internal standard. Blanks were also analysed during the extraction, isolation and analysis and contained negligible concentrations of stanones in the samples. 5 α -Cholestan-3-one was added to pre-extracted sediment in the extraction thimble and used to quantify stanone recoveries. Mass spectra were obtained using a Varian aerograph 1400 gas chromatograph equipped with an SE-52 glass capillary column (25 m $\times$ 0.30 mm i.d.) interfaced to a Finnigan 1015 C quadrupole mass spectrometer¹. The mass spectrometer

Steroids ketones in surface sediments from the south-west African shelf

STEROIDS are a class of biologically produced compounds which may serve as tracers for the transformation processes of labile organic matter in sediments. Diagenetic alteration of steroid alcohols (sterols) by geochemical and biological processes often leads to the accumulation of transformed products in the sediments. By elucidating these structures and determining their concentrations we should be able to determine their reaction mechanisms and rates of degradation. We have previously postulated that steroid ketones (stanones) are intermediates in

Their absence may be due to a more rapid rate of hydrogenation relative to their rate of formation from stenol oxidation. Hence their steady-state concentration would be quite low. Work is now being carried out on other sediment samples from both oxic and anoxic environments to elucidate further the proposed transformations outlined above. We emphasise, however, that the proposed mechanism in Fig. 1 is probably only one of several degradation pathways for sterols in sediments.

Pathways leading to the formation of sterenes from stenols have been proposed by several authors^{1,22,24-28}. These transformations in Recent marine surface sediments can be envisaged as a series of oxidation, reduction, and dehydration reactions. The sampling site off the south-west coast of Africa is an area of intense upwelling and high productivity. The presence of known microbiological products^{20,29} such as stanols³⁰ (C. Lee, unpublished results), stanones and sterenes¹ in the sediments and the rapid rate of sediment deposition in this sampling area suggests microbially mediated transformations. For example, cholest-4-en-3-one and 5 α -cholestan-3-one have been proposed as intermediates in the microbial reduction of cholesterol to cholesterol²⁹. However, little is known about the metabolism of marine bacteria, and much more information is required to understand more fully the processes influencing the transformation reactions of steroids in marine sediments.

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Geological evidence in favour of a Jotunheimen Caledonian suture

EARLY plate-tectonic reconstructions of the proto-Atlantic by Wilson¹ and Dewey² placed a Caledonian suture through central, southern Norway. Later, more detailed interpretations generally favoured a single (proto-Atlantic, Iapetus) suture off the west coast³⁻¹⁴, in agreement with the pre-plate-tectonic orthodoxy¹⁵⁻¹⁸ of a western, eugeosynclinal source for the far-travelled (up to 1,000 km) (ref. 13), crystalline and supracrustal nappes of Norway and Sweden. In South Norway, however, not all interpretations fitted this pattern. Goldschmidt²¹ and Oftedahl²² proposed a local root zone in the Jotunheim for the crystalline Jotun Nappe south-east of its present northwesterly margin. More recently Battey and McRitchie^{23,24} demonstrated a close fit between surface geology and the interpretations of gravity data by Smithson *et al.*²⁵ of the Jotun Nappe as an 'Ivrea type' flake with a local root possibly as deep as 16 km. Note that recent⁴³ aeromagnetic results suggest a maximum thickness of only 5 km for the Jotun Nappe). Evidence from the northwestern margin of the Jotunheim is clearly critical, for the south-eastward transport of the Jotun Nappe deduced from areas to the south-east^{14,18} is consistent with derivation from both the Iapetus suture and a possible Jotunheim suture. Some reports, however, apparently support the Jotunheim suture hypothesis. Between Fjaerlandsfjorden and Sogndalsdalen, Skjerli²⁶ found imbricated thrust slices of Basal gneisses and supracrustal rocks indicating transport to the north-west. A similar conclusion was reached by Battey and McRitchie²³ for Bøverdalen. Also, Elliott and Cowan²⁷ describe a sequence of tholeiitic greenstones with pillows and serpentinites from Holleindalen in Bøverdalen. Most recently, however, Roberts⁴² has concluded, using structural evidence from seven traverses across the northwestern margin, that the Jotun Nappe was transported to the south-east. The work described here does not support this conclusion.

Within the Sygnefjell area (Fig. 1) an Assynt-type nappe culmination permits the examination of an extensive sequence of supracrustal rocks between the Basal gneisses and the overlying Jotun Nappe. Six main lithostructural units have been identified:

- | | |
|-------------------------------------|------------------------------|
| 6 Jotun mangerites and anorthosites | } Jotun Nappe;
allochthon |
| 5 Galdeberghytta gneisses | |
| 4 Helgedalen metagreywackes | } Parautochthon? |
| 3 Turtagrø metavolcanics | |
| 2 Sygnefjell sparagmites | |
| 1 Basal gneisses | Autochthon |

The Basal gneisses form part of the extensive northwestern Basal gneiss complex of South Norway. An early suite of

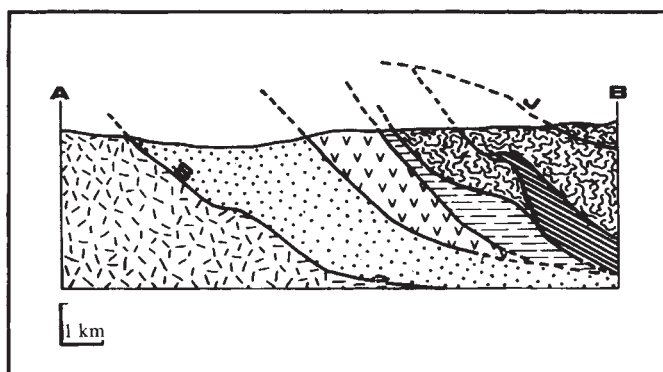


Fig. 1 A simplified lithostructural map of the Sygnefjell area: 1, Basal gneisses; 2, Sygnefjell sparagmites; 3, Turtagrø metavolcanics; 4, Helgedalen metagreywackes; 5, Galdeberghytta gneisses; 6, Jotun mangerites and anorthosites. St, Storevatnet; Sk, Skaalavatnet; Pr, Prestesteinsvatnet; H, Helgedalen; G, Galdeberghytta; K, Krossbu.

basic-intermediate rocks has been migmatized and metamorphosed in the almandine-amphibolite facies and intruded by foliated granites dated at 1,033 Myr (refs 28, 29).

Micaceous meta-arkoses (sparagmites) have, in places, a basal conglomerate resting unconformably on the Basal gneisses. They are intercalated with metalimestones, graphitic micaschists and hornblende schists higher in the sequence.

The Turtgrø metavolcanics comprise some 600 m of andesitic and basic lavas, some with pillows, and tuffs within a thrust bounded slice. This is overlain by up to 1 km of Helgedalen metagreywackes. The greywackes grade from a proximal, quartz-rich, conglomeratic facies at the base, to a distal, finegrained, apparently volcanoclastic facies towards the top. Graded bedding and various other sedimentary structures indicate deposition by turbidity currents, and provide evidence that the unit as a whole is the right way up.

All supracrustal units have been recrystallised in the green-schist facies. The absence of fossils or radiometric dates precludes a reliable assessment of their ages, although regional considerations^{18,42} suggest an Eocambrian to Lower Ordovician age. Despite the thrusts within and between these supracrustal units, all are at present regarded as parautochthonous in view of the apparent lithological gradations between them. Moreover, all these units are intruded by pre-kinematic, minor gabbros, dolerites and diorites.

The Galdeberghytta gneisses at the base of the Jotun Nappe are partially mylonitized, amphibolitic and augen gneisses with K-feldspar-rich, late pegmatites and minor intrusives. The upper part of the Jotun Nappe consists of layered gabbros, leucogabbro, anorthosite and mangerites in various stages of retrogression from granulite facies, pyroxene-bearing assemblages. Battey and McRitchie²³ cite K-Ar age determinations of up to 1,280 Myr for these rocks to the south-east of the Sygnefjell area.

Structural work has shown that both the Basal gneisses and the Jotun Nappe rocks were deformed and metamorphosed before the events that affected the supracrustal sequence. The supracrustal rocks share a common polyphase structural sequence. A transposed tectono-metamorphic banding was developed to varying degrees in all units during early deformations (D_1 and D_2). Where younging criteria can be identified, the D_1 folds face the north-west, suggesting relative transport in that direction. Intense flattening in the limbs of the D_2 isoclinal folds has resulted in discrete zones of mylonitic rocks. Following D_2 , a phase of upright asymmetric folding (D_3) with the steep limbs overturned to the north-west took place syntectonically with major brittle thrusting (D_4) of all units (Fig. 2). The thrusting shows classical imbrication patterns, with the thrusts climbing section towards the north-west, indicating transport in that direction.

A similar structural geometry and history of folding has been described by Hopper (in preparation) from Østerøy near the northeastern margin of the Bergen Arcs, and by Cowan³¹ from Bøverdalen. The Vassenden area, Bøverdalen, is one of those recently described by Roberts⁴². Both Cowan's (ref. 31 and personal communication) earlier work at Vassenden, and evidence from the nearby Sygnefjell area, indicate that a major phase of structures, including north-west facing folds (our D_1), pre-dated the D_1 of Roberts. Thus, in our view, the Vassenden area does not provide good evidence for nappe transport from the north-west.

An outline of the sequence of events in the Sygnefjell and nearby areas together with suggested broader interpretations can now be given.

First, pre-Sveconorwegian^{23,29,32} high grade metamorphism and deformation of Jotun Nappe rocks (and of the north-west Basal gneisses and southern gneisses of other areas^{29,34,38}).

Second, Sveconorwegian (1,033 Myr) intrusion of Hestbrepiggen granite into Basal gneisses.

Third, post-1,033 Myr (Eocambrian to Lower Palaeozoic?): (1) deposition of a fining-upwards sequence of conglomerates, limestone and shale; (2) sub-aqueous basaltic volcanism (ocean

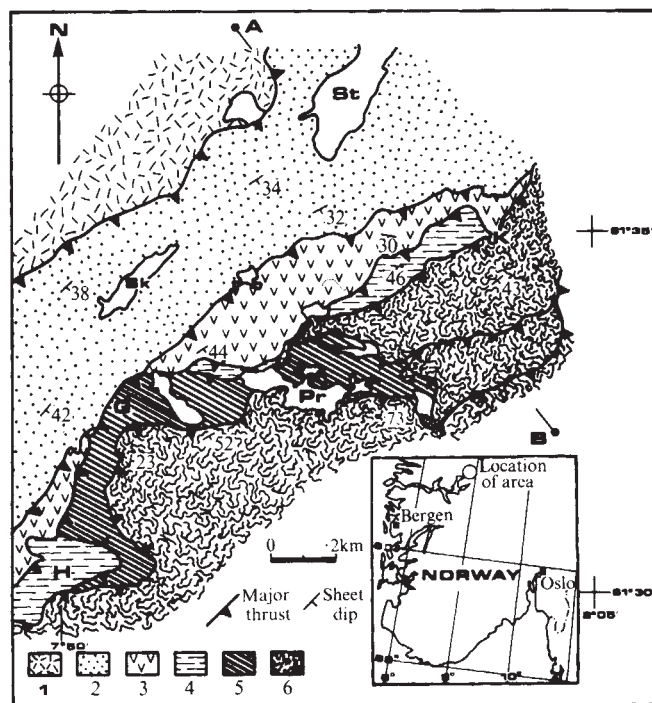


Fig. 2 A simplified dip section of the Sygnefjell area; the area has an along-strike relief of 1.3 km. J, Jotun Thrust; S, Sole Thrust; A-B, ornament as on Fig. 1.

spreading); (3) andesites and turbiditic, volcanoclastic greywackes (island arc-trench system); (4) greenschist facies metamorphism and deformation of supracrustal wedge (ocean closure); (5) thrusting of the Jotun Nappe to the north-west (after flaking from lower crust during final stages of ocean closure); (6) broad warping and sinistral wrench faulting (after ocean closure).

Long distance southeastward transport of the Jotun Nappe has been suggested in the past for two main reasons. First, on the similarity of the Jotunheim and Bergen Arc high-grade meta-plutonic rocks. Deep crustal granulite facies rocks are not uncommon in old basements, however, and many Jotun Nappe rocks can be matched in the basements both to the north-west and to the south-east of Jotunheimen (see refs 33, 39, 40). In any case, lithological similarity alone cannot indicate the direction of nappe transport.

Second, the occurrence in the nappe region of east to south-east trending penetrative lineations has been taken (see ref. 16) to indicate a southeastward direction of nappe transport. In the Sygnefjell area the early, penetrative lineation is due to the superimposition of at least two strains (D_1 and D_2) and a transport direction cannot be deduced from this evidence alone. Similar conclusions have recently been reached in the Bergen area by Faereth *et al.*⁴¹ and one of us (F.W.M.H., in preparation).

Within the Sygnefjell area, two groups of observations are consistent with the hypothesis of a Jotunheim suture:

First, the facing directions of the early folds (D_1), and, especially, the thrust geometries produced during the main movement phases (D_4), indicate tectonic transport to the north-west.

Second, the occurrence of a probably parautochthonous, oceanic, supracrustal sequence of arkoses, turbiditic greywackes, serpentinites and basaltic and andesitic volcanics.

On the other, southeastern margin of Jotunheim, it is well-established that both the supracrustal and the crystalline nappes were transported towards the south-east. That the supracrustal nappes moved considerable distances (100–270 km) is shown by Hossack's¹⁴ recent unravelling of the thrust stack. The even greater distance of transport (290+ km) 'assumed' (ref. 14 p 239) for the crystalline, Jotun Nappe above, although consistent

with evidence from the supracrustal nappes, is not required by it, as Hossack clearly recognises. When the Sygnefjell evidence is also considered, a possible interpretation emerges of the Jotun Nappe as a flake detached from local, lower crust and thrust over (underthrust by) gneissose basements and marginal sedimentary basins both to the north-west (such as Sygnefjell) and to the south-east (such as Bygdin). The presence of igneous rocks within the northwestern supracrustals may indicate their proximity to the site of an active trench.

Further work will test the hypothesis of a Jotunheim Caledonian Suture. The structural evidence reported here will be published in full elsewhere.

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Mechanism of reaction in metal + metal oxide systems

PYROTECHNICS frequently consist of intimate mixtures of powdered metals and metal oxides. The exothermicity of such reactions is often very high, as, for example, in the thermite process. Considerable self-heating occurs and the reaction often becomes explosive. Little experimental work has been carried out on the mechanism of these reactions, largely because the participation of solid phases and the high evolution of heat make them difficult to study. It is to be expected, however, that such mechanisms would normally involve solid phases, with the possible addition of a liquid phase when a low melting point reactant or intermediate is present. We report here that such reactions often occur through a gas phase process. Furthermore, when one of the constituents is magnesium, which is frequently the case in pyrotechnics, the reaction rate is controlled almost solely by the evolution of magnesium vapour.

The classical method, developed by Wagner¹ and by Tubandt²⁻⁴, for elucidating the identity of the mobile species and of any intermediate compounds formed, is to allow pressed pellets of the two separate solids to react together. The movement of the phase boundaries and the growth of intermediate zones of different composition then provide the required information. During preliminary studies on metal + metal oxide systems, using this technique, it was discovered that reaction proceeds normally even when there is a substantial gap between the two solids, and the system is well below the melting point of the materials involved. This demonstrated that the mobile species was present in the gas phase and the chemical reaction involved both gaseous and solid components. A more comprehensive series of experiments was then carried out.

A typical reaction chamber consisted of a pair of mild steel 'crucibles', each of 8 mm internal diameter and 8 mm internal depth. The reactant samples were ground and sieved to the required particle size and quantities of about 0.2 g were packed into each crucible to the required pressure. The crucibles were heated separately *in vacuo* to remove occluded gases and were then handled only in dry, O₂-free conditions. After weighing, the crucibles were clamped together so that the reactant surfaces were about 2 mm apart and the complete reaction chamber was heated in a thermostatted oven, either *in vacuo* or in a low pressure of argon, for the required period (typically 1 h). At the end of the experiment, the two crucibles were separated and re-weighed to determine the mass of material transported (typically ~40 mg, corresponding to 2 mmol of reaction or 20% conversion). The product layers were then separated manually for analysis. Blank experiments were also carried out on the individual components.

The main results are shown in Table 1, from which the most

Table 1 Summary of metal + metal oxide reactions

System	$\Delta H_{f,300}^0$ of oxide (kcal mol ⁻¹)	$\Delta H_{f,300}^0$ per atom of oxygen	Temperature of reaction (°C)	Mobile species	Products identified
Mg + SiO ₂	210	105	625	Mg	MgO, Si, Mg ₂ Si
Mg + Cr ₂ O ₃	275	91	625	Mg	MgO, Cr, MgCr ₂ O ₄
Mg + MoO ₂	133	66	625	Mg	MgO
Mg + WO ₂	137	69	625	Mg	
Mg + B ₂ O ₃	302	101	1000*	Mg	MgO, Mg B ₂
Mg + Al ₂ O ₃	404	135	625	Mg	MgO, Al
Mg + TiO ₂	228	114	625	Mg	MgO, Ti
Mg + CuO	37.7	37.7	625	O	MgO, Cu
Mg + NiO	57.5	57.5	625	O	MgO, Ni
Ti + Cr ₂ O ₃	275	91	1300		no reaction
Ti + NiO	57.5	57.5	900	O	Ni, TiO ₂

The heat of formation, $\Delta H_{f,300}^0$, for MgO = 144 kcal mol⁻¹.

* The amount of reaction at lower temperatures was negligible.

important conclusion is that, in the majority of cases involving magnesium, which has a vapour pressure of ~ 2 torr at these temperatures, the mechanism simply involves evaporation of magnesium from the solid followed by reaction at the oxide surface, in one or more stages. In such cases, the rate is determined primarily by the rate of evaporation of magnesium, an exception being the reaction with boric oxide.

When the oxide is less stable, as indicated by a lower heat of formation, the mobile species becomes oxygen. However, the mechanism involved is not simple dissociation of the oxide, for the following reasons. (1) In the absence of the metal reactant, the weight loss from the oxide was negligible (as would be predicted from the dissociation pressures). (2) In the presence of a non-volatile metal, the reaction was much slower and required a substantially higher temperature. (3) The extent of reaction was comparable with that of systems in which magnesium was transported. (4) Some of the magnesium oxide ($\sim 5\%$) was detected on the Ni/NiO surface.

It seems, therefore, that the dissociation of the oxide to give mobile oxygen is 'catalysed' by the presence of metal, presumably due either to the considerable heat evolved on reaction, thus raising the temperature of the oxide surface, or to the formation of intermediates of different composition.

Attempts to compare the kinetics of reaction using this technique were of only limited value for two reasons: first, the initial rate of evaporation of magnesium depended very much on its pretreatment and was thus rather irreproducible, and second, because the rate decreased markedly with time, due presumably to inhibition by solid product, the extent of reaction tended to be similar even when the initial rates differed. Quantitative kinetic studies will require a controlled and measurable pressure of magnesium vapour.

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Photosynthesis in some British coastal waters may be inhibited by zinc pollution

RIVER drainage from mineral-bearing and industrialised regions has produced elevated concentrations of heavy metals in several parts of the coastal waters around the UK, including Liverpool Bay^{1,2}, Cardigan Bay¹, the Bristol Channel¹⁻³, off the mouth of the River Tees⁴ and in the Firth of Clyde⁵. Little attention has been paid, however, to the possibility that the presence of the metals might be affecting the planktonic marine life of these sea areas. Phytoplankton represent the basic food resource on which the majority of marine animals and fish directly or indirectly depend, and it is thus conceivable that inhibition of their growth by toxic metals could have repercussions throughout the local marine food webs. We are investigating the role of trace quantities of both biologically

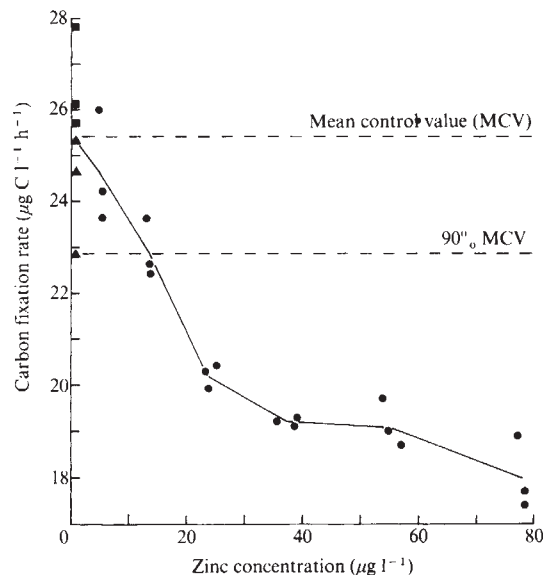


Fig. 1 The effect of increasing concentrations of zinc on the rates of carbon fixation in the phytoplankton present at Station L4 ($50^{\circ}15' \text{ N}$, $4^{\circ}13' \text{ W}$) in the English Channel on 10 July 1978. The lines connect the mean values of each group of results. The measurements were carried out at 14°C and 10 klx of illumination by Northlight fluorescent lighting. Sample 1 contained $2.1 \mu\text{g}$ chlorophyll *a* per l, the phytoplankton being almost exclusively diatoms of the genus *Rhizosolenia*. The chlorophyll *a* concentration has been corrected for the presence of phaeo-pigments.

▲, ■, Controls.

beneficial and toxic substances in regulating the 'quality' of seawater, that is, its capacity to support and maintain, through photosynthesis, the production of living matter from the inorganic nutrients present in the water. As part of this research programme, we have examined how carbon fixation rates in natural populations of phytoplankton are affected by zinc concentrations encompassing those observed in the areas mentioned. Although zinc is a component of many enzyme systems⁶ and therefore essential for the growth of phytoplankton⁷, it becomes toxic if present greatly in excess of their basic requirements. We report here data which suggest that phytoplankton growth could be depressed by the zinc concentrations present in some British coastal waters.

Full details of the techniques being used will be published elsewhere. Briefly, samples of surface seawater, strained through a $170\text{-}\mu\text{m}$ nylon mesh to remove the zooplankton, were rapidly transported from the point of collection—Station L4 about 10 km south west of Plymouth breakwater—to our laboratory. During the journey, the samples were kept at seawater temperature and exposed to natural lighting conditions. Immediately after their arrival in mid-afternoon, zinc labelled with ^{65}Zn was added to eight 1-l subsamples of the water to give initial concentrations ranging from those present naturally (in the two controls) up to about $90 \mu\text{g l}^{-1}$. The subsamples were then placed in an illuminated incubator maintained at the temperature of the seawater at the time of collection and left there overnight to allow the added zinc to equilibrate with the plant cells. The water was aerated throughout the whole of this period in the laboratory to maintain a constant pH and keep the phytoplankton in suspension. Between 20.00 and 06.00 h, the lights were extinguished to simulate overnight darkness. The next day, the carbon fixation rates of the phytoplankton in each of the subsamples were determined in triplicate in the illuminated incubator by measuring the rate of incorporation of $^{14}\text{CO}_2$ (ref. 8). Discrimination between the β and γ radioactivities due to the ^{14}C and ^{65}Zn , both in the water and in the phytoplankton (after their removal from the water by membrane filtration), allowed the carbon fixation rates and the amounts of zinc taken up by the plant cells (expressed as $\mu\text{g Zn}$

per μg chlorophyll *a*) to be determined as functions of the concentration of zinc present in the water during the carbon fixation measurement. The zinc concentration at this stage of the experiment was always lower than that initially, due to adsorption on to the glassware used to contain the water samples.

Figures 1 and 2 show the results of the measurements on one of the samples. Two further samples collected on successive weeks contained phytoplankton populations consisting of approximately equal numbers of dinoflagellates (mainly *Scip-sip-siella* aff. *trochoidea*) and diatoms (mainly *Rhizosolenia* spp.); carbon fixation rates in these were similarly depressed by the presence of zinc. In a control experiment, the normal routine was carried out with the exception that zinc was not added to any of the eight subsamples. In this case, the carbon fixation rates in each of the 24 final determinations agreed to within $\pm 10\%$ of the mean value. In Fig. 1, fixation rates less than 90% of the mean control value can thus be regarded as due to perturbations caused by the zinc.

The results show that zinc concentrations down to $15 \mu\text{g l}^{-1}$, and possibly lower, inhibited photosynthesis in the phytoplankton populations present in the English Channel in July 1978. The degree of inhibition was, however, a nonlinear function of the zinc concentration and above about $30 \mu\text{g l}^{-1}$, there was very little further decrease in the carbon fixation rates. This was apparently due to the near saturation of the plant cells with zinc at the higher concentrations, as the zinc/chlorophyll *a* ratios in the phytoplankton were then approaching their maximal values (Fig. 2); the forms of these zinc sorption isotherms were found to differ from one sample to the next, probably due not only to the change in the species composition of the phytoplankton present in the water but also, to some extent, to uptake on to detrital matter which it was not possible to separate from the plant cells. These observations support the recently expressed view⁹ that the effect of metals on the growth of phytoplankton is more closely related to the amounts accumulated by the plant cells than to the concentrations in the water surrounding them.

In the contaminated sea areas, dissolved zinc concentrations between 5 and $10 \mu\text{g l}^{-1}$ were found to be widespread and at many locations, sometimes greatly, exceeded $10 \mu\text{g l}^{-1}$ (refs 1-3, 5). Thus, it seems possible that primary production in these regions may already have been depressed as the result of metal pollution, particularly when it is remembered that zinc was just one of a range of metals usually present in the water¹⁻⁵, and synergistic effects as observed with zinc and copper mixtures in laboratory cultures¹⁰ could amplify their individual toxicities towards the phytoplankton.

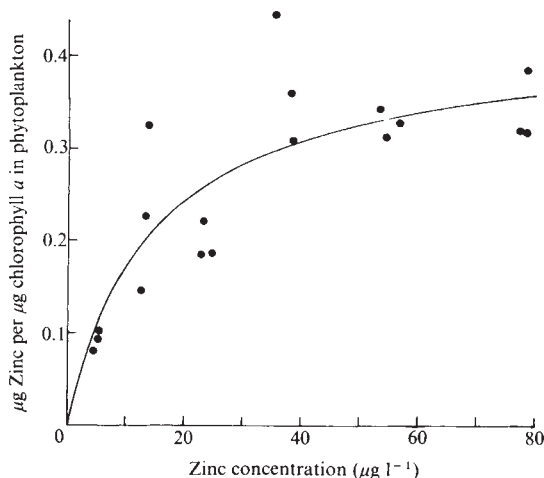


Fig. 2 Zinc/chlorophyll *a* ratios in the phytoplankton during measurement of the carbon fixation rates in relation to the zinc concentration in the seawater. The curve represents the best fit of the Langmuir adsorption isotherm $y = Ax/(B + x)$ to the data, the values of *A* ($\mu\text{g Zn per } \mu\text{g chlorophyll } a$) and *B* ($\mu\text{g Zn l}^{-1}$) used to compute it being 0.42 and 14.7.

However, because our measurements were short term and relate to phytoplankton growing in the relatively unpolluted waters of the English Channel, extrapolation of the present data to other sea areas can only be made with some reservations. First, the metal contamination is generally associated with river drainage so that the polluted seawater is also likely to contain higher than usual levels of dissolved organic matter¹¹. The formation of metal-organic complexes would decrease the biological availability of the metals and hence the amounts taken up by the phytoplankton, thereby moderating their effects in the way that chelating agents reduce the toxicity of copper in phytoplankton cultures¹². Second, chronic exposure of metal-sensitive phytoplankton populations to sublethal metal concentrations might eventually lead to their replacement by tolerant species or the development of resistant strains¹³ which would be unaffected by the presence of toxic metals. Nevertheless, the existence of potentially harmful zinc concentrations in our coastal waters clearly requires further investigations.

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Widespread occurrence of a unicellular, marine, planktonic, cyanobacterium

IN marked contrast to their freshwater counterparts, marine planktonic cyanobacteria are restricted to a few nostocalean genera, of which only *Trichodesmium* is capable of forming extensive water blooms¹⁻³. We report here the widespread occurrence of a small, marine, chroococcoid cyanobacterium belonging to the genus *Synechococcus*.

Natural water samples were filtered through $0.2 \mu\text{m}$ Nuclepore filters, counterstained with Irgalan black⁴. The filters were examined with a Zeiss Standard microscope equipped with Neofluar objectives and an epifluorescent illumination system containing a 100-W halogen lamp, a BP 450-500 excitation filter, a LP 528 barrier filter and a FT 510 chromatic beam splitter. Using this system, phycoerythrin-containing cyanobacteria fluoresce orange and can be distinguished from phytoplankters that fluoresce red.

Phycoerythrin-rich unicellular cyanobacteria were observed at seven stations in the Arabian Sea in January 1977, at three stations off the coast of Peru in March 1978, in Slope Water north of the Gulf Stream in April 1978, and periodically in Woods Hole Harbor. In the relatively rich waters of the Arabian Sea and off the coast of Peru, the population varied from 10^4 to 10^5 cells ml^{-1} within the euphotic zone (Table 1). The greatest number of cells was found within the top 20 m of the water column, with occasional cells being observed as deep as 400 m. In contrast, the surface sample collected from Slope Water north

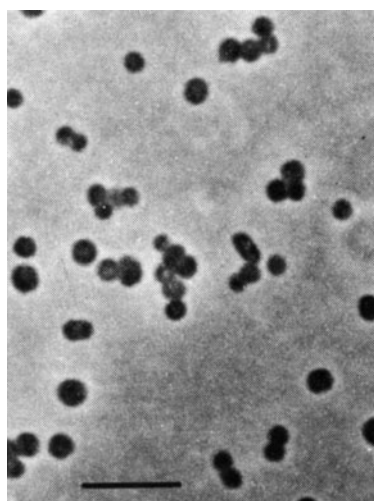


Fig. 1 Phase contrast photomicrograph of *Synechococcus* sp. (strain Syn-48) illustrating general cell morphology (scale bar, 5.0 μm).

of the Gulf Stream contained 1.3×10^3 cells ml^{-1} . In Woods Hole Harbor, counts have ranged from 2×10^3 cells ml^{-1} on 1 May 1978 to 3.6×10^5 cells ml^{-1} on 1 June 1978, at which time 15–20% were in a late stage of binary fission.

No attempt was made to culture the cyanobacteria from the above locations. However, in 1965, one of us (R.R.L.G.) isolated a small, red, coccoid cyanobacterium (strain Syn-48) off the north-east coast of South America ($08^{\circ}44' \text{ N}$, $50^{\circ}50' \text{ W}$) with morphological and fluorescent properties similar to the cyanobacteria observed in open ocean waters by epifluorescence microscopy. Its size is $0.9\text{--}1.3 \times 1.8\text{--}2.2 \mu\text{m}$ and it divides in one plane (Fig 1). Examination of thin sections by transmission electron microscopy reveals that it has a typical synechococcoid ultrastructure, with the thylakoids arranged peripherally. H. W. Siegelman (personal communication) reports that its pigment system is typical of other cyanobacteria, containing chlorophyll *a* as its major photosynthetic pigment and phycobiliproteins as accessory pigments. It always appears red in culture, due to both a predominance of phycoerythrin, one of the accessory photosynthetic pigments, and its inability to adapt chromatically^{5,6}. The strain was isolated from a depth of 75 m where the salinity was 35.87‰. Preliminary growth experiments, carried out as described by Hargraves and Guillard⁷, reveal that it will not grow at salinities below 14‰, indicating that it is intrinsically marine^{8,9}.

Recently, several additional strains of small, red, coccoid cyanobacteria have been cultured from Georges Bank and the Sargasso Sea. In general, they resemble both strain Syn-48 and

the cells observed by epifluorescence microscopy. Individual isolates vary from 0.8 to 2.0 μm in diameter following binary fission.

Cell size and shape accompanied by division in one plane indicate that these cyanobacteria are assignable to the genus *Synechococcus*¹⁰, an assignment which is further substantiated by the typical synechococcoid ultrastructure of strain Syn-48. The range in cell size among the isolates now in culture suggests that they may represent more than one species.

The use of epifluorescence microscopy to visualise the natural orange fluorescence of phycoerythrin seems a useful technique to enumerate small, phycoerythrin-containing unicellular cyanobacteria. However, not all cyanobacteria contain phycoerythrin, so that the specific set of filters used in this study cannot be used to count unicellular cyanobacteria in general. Furthermore, phycoerythrin is not unique to cyanobacteria but is present in almost all red algae (Rhodophyta) and in many cryptomonads (Cryptophyta). In principle, members of the latter two groups could interfere when attempting to count unicellular cyanobacteria, but in practice differences in size and chloroplast morphology should permit the unambiguous distinction between these eukaryotic algae and unicellular cyanobacteria.

Cyanobacteria have an ancient marine history¹¹ and show a considerable diversity and abundance in other marine habitats (for example, the intertidal zone)^{1,2}. The existence of small unicellular cyanobacteria with a widespread geographical distribution and the ability to achieve considerable cell densities represents the second example of a planktonic cyanobacterium that has adapted to growth in the open ocean and the first well documented member of the Chroococcales to do so. The ecological importance of these cyanobacteria as major primary producers in the world's oceans is still to be assessed.

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Table 1 Representative vertical distributions of *Synechococcus* sp.*

Depth (m)	Station 28 Peru 13°52' S, 76°34' W	Station 30 Peru 13°57' S, 77°10' W	Station 2354 Arabian Sea 21°16' N, 68°05' E	Station 2356 Arabian Sea 24°12' N, 64°44' E
0	1.90	8.80	12.00	5.30
10	2.60	1.08	12.50	
20	0.48	1.04	4.28	
25				5.28
40	0.024	0.076		
50			6.72	3.95
60				
75			6.08	
100		0.16	3.00	0.016
150			0.016	
200		0.026	0.013	0.013
300				0.011
400			0.010	<0.001

* 10^4 Cells per ml.

Pollen dispersal and optimal outcrossing in *Delphinium nelsoni*

NATURAL SELECTION, in sexually reproducing plants, should often favour matings between individuals of intermediate genetic similarity. Matings between very similar individuals may lead to inbreeding depression because segregational load is revealed^{1,2}, while matings between very dissimilar individuals may disrupt favourable gene combinations and lead to outbreeding depression^{3–5}. Outbreeding depression in plants has

been documented in crosses between species, varieties and isolated populations⁶⁻⁹, and reports of inbreeding depression date back at least a century¹⁰. We suggest that outbreeding depression will often occur on a much finer scale than previously recognised, especially in plants subject to restricted pollen and seed dispersal. Such plants are likely to show pronounced microgeographic genetic differentiation resulting from drift in subpopulations isolated by distance or from adaptation to local edaphic and biotic conditions^{11,12}. Under these circumstances, a short outcrossing distance may be optimal not only because of intragenotypic effects, but also because it produces offspring sufficiently similar to the female parent to grow successfully near her, yet sufficiently genotypically diverse to maximise success of the total progeny in the face of coarse-grained temporal environmental variation¹³⁻¹⁵, frequency-dependent sibling competition¹⁶⁻¹⁸ or predation¹⁹⁻²⁰. Here we present evidence that a short outcrossing distance is optimal for *Delphinium nelsoni* Greene and discuss the relationship between the optimal outcrossing distance for *D. nelsoni* and actual pollen dispersal by its main pollinators.

D. nelsoni is an herbaceous perennial which flowers in early summer around the Rocky Mountain Biological Laboratory (RMBL) in western Colorado²¹. It lacks clonal spread, the flowers are usually borne 10–20 cm from the ground and seeds drop an average of 11.2 cm from the central stalk. To investigate the effects of outcrossing distance on fitness, we hand-pollinated 1,016 flowers on 317 representative *D. nelsoni* plants near

Table 1 Effects of outcrossing distance on seedling survivorship in *D. nelsoni*

	Selfed	1 m	Treatment 10 m	100 m	1,000 m
1977 Meadow 1 seeds, planted in Meadow 1, censused after 1 yr					
No. planted	58	—	145	—	116
No. surviving	3	—	23	—	13
Proportion surviving	0.052	—	0.159	—	0.112
1976 meadow 1 seeds, planted in Meadow 2, censused after 2 yr					
No. planted	98	98	98	98	98
No. surviving	0	3	7	1	1
Proportion surviving	0	0.031	0.071	0.010	0.010

A significantly higher proportion of 10 m seeds survived overall than either 1,000 m (d.f. = 4, $\chi^2 = 13.47$, $P < 0.01$) or selfed (d.f. = 4, $\chi^2 = 28.30$, $P < 0.005$) seeds, using combined probabilities from separate one-tailed *t* tests of differences of proportions with each replicate²².

RMBL in 1976, 1977 and 1978. In the 1976 Meadow 1 and 1977 Meadow 2 experimental replicates, we bagged recipient plants to exclude natural pollinators and chose pollen donor flowers either from the same plant (self pollination) or from plants 1, 10, 100 or 1,000 m distant. In the 1977 Meadow 1 replicate we omitted 1 and 100 m treatments, while in the 1978 Meadow 1 replicate we substituted 3 and 30 m for selfed and 1,000 m treatments. Different pollen donor populations were used for Meadows 1 and 2, which are separated by 300 m of aspen forest and differ by 60 m elevation. We assigned each plant at random to a single distance treatment to avoid systematic bias in plant size or ovule number per flower, and used individual pollen loads from that distance to pollinate several of its flowers as they became receptive. Pollen loads were collected on circular toothpicks, usually from one donor flower but where dehiscing anthers were scarce from as many as three flowers on two plants. This collection technique was consistent between treatments within a replicate. In the 1977 Meadow 2 and 1978 Meadow 1 replicates, we equalised the amount of mechanical handling of pollen during transport from the donor population and the total elapsed time between its collection and deposition across treatments. We harvested fruits on recipient plants just before dehiscence and counted all fully developed seeds. Our hand pollination procedure consistently resulted in seed sets of approximately 15–35% of open-pollinated averages²¹. In all replicates, we left several flowers per plant unpollinated to serve as controls for failure of bags to exclude pollinators. We discarded the few plants in which these control flowers set copious seed, as *D. nelsoni* is protandrous and rarely sets seed when pollinators are effectively excluded²¹. In the 1976 Meadow 1 replicate, we lumped all mature seeds for each treatment and planted them in a 10 m² plot near Meadow 2. In the 1977 Meadow 1 replicate we lumped mature seeds from each plant and planted each progeny separately within a 2 m² plot in the center of Meadow 1. These plots were censused in early June during subsequent summers to determine seedling survivorship.

For all hand pollination experiments mean seed sets per flower were highest with an intermediate outcrossing distance and declined significantly at longer and shorter distances (Fig. 1). The three 1976–77 replicates consistently indicate an optimal outcrossing distance of 1–100 m, whereas the 1978 replicate more precisely indicates an optimal distance between 1 and 10 m. Note that in Meadow 1 mean seed sets for each treatment were especially similar between years. An optimal outcrossing distance between 1 and 100 m again appeared in seedling survivorship in natural meadows, and inbreeding and outbreeding depressions in survivorship were also significant (Table 1).

The main pollinators of *D. nelsoni* in the RMBL area are broad-tailed hummingbirds (*Selasphorus platycercus*), occasional rufous hummingbirds (*S. rufus*) and bumblebees (primarily *Bombus appositus* and *B. flavifrons* queens)²¹. Pollen dispersal by these visitors was estimated in two ways in 1976: (1) Foraging pollinators were followed in Meadows 1 and 2, with observations of bees and birds matched by meadow, day, and

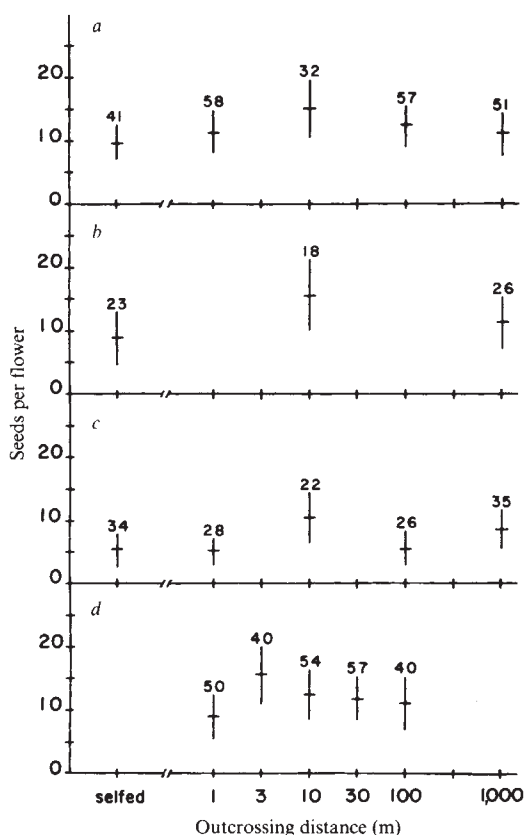


Fig. 1 Effects of outcrossing distance on seed set in hand-pollinated *D. nelsoni*. a, Meadow 1, 1976; b, Meadow 1, 1977; c, meadow 2, 1977; d, meadow 1, 1978. Values are means $\pm$ 95% confidence units, with sample sizes shown. Two statistical tests establish significance of the intermediate outcrossing optimum. First, for the three 1976–77 experimental replicates, 10 m seed sets were significantly higher overall than either 1,000 m (d.f. = 6, $\chi^2 = 12.59$, $P < 0.05$) or selfed (d.f. = 6, $\chi^2 = 24.20$, $P < 0.005$) seed sets, using combined probabilities from separate one-tailed *t* tests with each replicate²². Second, the probability of observing by chance alone an intermediate optimal outcrossing distance in the 1976 replicate, followed by peaks at the same distance interval for the remaining three replicates is $3/5 \times 1/3 \times 1/5 \times 3/5 = 0.024$.

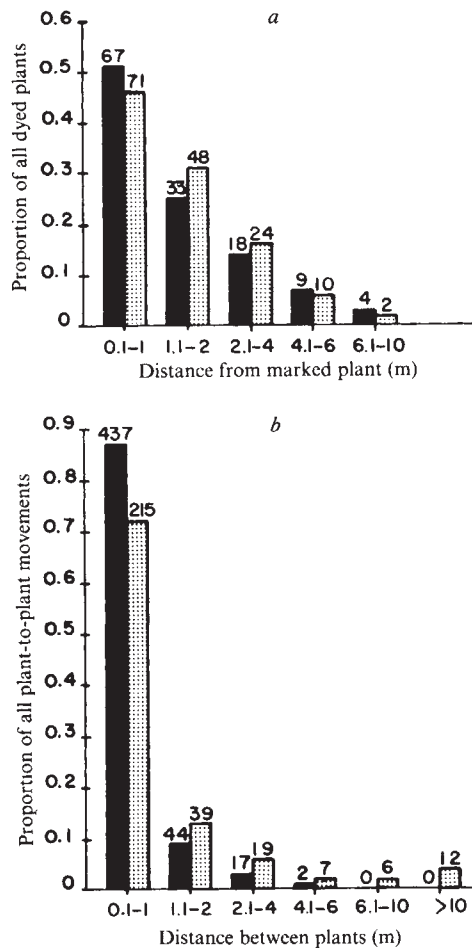


Fig. 2 Estimates of pollen dispersal by pollinators of *D. nelsoni*. *a*, Distributions of dye dispersal distances for caged (■) ($\bar{x} = 1.64$ m) and uncaged (□) ($\bar{x} = 1.57$ m) plants, with sample sizes (numbers of neighbouring plants receiving dye) shown. *b*, Distributions of plant-to-plant movement distances for hummingbirds (□) (from 19 foraging bouts $\bar{x} = 2.1$ m) and bumblebees (■) (from 12 foraging bouts $\bar{x} = 0.63$ m), with sample sizes (number of movements) shown above each histogram.

time of day, and numbers of flowers visited per plant and distances flown between successive plants were noted. Densities in these meadows averaged 16 plants per m^2 . (2) Dispersal of fluorescent pigments dusted on 18 plants in Meadow 2 was measured. Half of these plants were caged with chicken wire to exclude birds but not bees, and half were exposed to both pollinators. Caged and uncaged plants were dusted in pairs with distinct colours over a period of 2 weeks. Three to five days after dusting, neighbouring plants within a 10 m radius were sampled and scored for presence of pigment grains on floral parts contacted by pollinators.

Figure 2 compares distributions of plant-to-plant movements of hummingbirds and bumblebees and patterns of pigment dispersal from caged and uncaged plants. On average, birds moved 3.4 times as far as bees, but pigment moved no further from uncaged plants (exposed to birds as well as bees) than from caged plants. Movement distributions for bees and birds differ significantly overall ($\chi^2 = 21.0$, d.f. = 5, $P > 0.005$), while those for pigment from caged and uncaged plants do not ($\chi^2 = 2.5$, d.f. = 4, $P > 0.5$). Movement distributions for both pollinators are more highly leptokurtic than those for pigment, perhaps as a result of pigment carryover past the first recipient flower.

Expressing pollinator foraging only in terms of plant-to-plant movements conceals the fact that hummingbirds visit more flowers per plant than do bumblebees (birds, 2.5 ± 0.09 flowers per plant (296); bees, 1.4 ± 0.06 flowers per plant (502); values are means $\pm$ one s.e.m. followed by sample sizes). Counting

within-plant movements as 0 m, mean flower-to-flower distances flown by the two pollinator types are more similar than mean plant-to-plant distances (birds, 0.8 ± 0.13 m between flowers (741); bees, 0.4 ± 0.03 m between flowers (731). Within-plant movements of hummingbirds are also especially likely to lead to selfing: birds appear to move unsystematically between flowers on an inflorescence, whereas our observations suggest that bees usually visit lower, pistillate flowers before moving up an inflorescence to staminate flowers^{23,24}.

The mean pollen dispersal distance estimated by these methods is about 1 m, the lower end of the 1–10 m range we construe from hand pollination to contain the optimal outcrossing distance for *D. nelsoni*. This discrepancy may indicate our inability to measure pollen flow directly or to define optimal outcrossing precisely using hand pollination. On the other hand, we do not necessarily expect to find congruence between actual pollen flow and that which is optimal from the point of view of female function. Instead, actual pollen flow can be expected to reflect: (1) conflicting selection on plants to maximise amounts of pollen received as well as to optimise transfer distances (for example, bees and birds may move more pollen than birds alone even though birds move slightly longer distances); (2) conflicting selection on plants to donate as well as receive pollen over optimal distances (for example, optimal dispersal distances may differ for male and female functions); or (3) conflicting selection on pollinators to minimise flight distances and on plants to achieve optimal outcrossing. In (3) it is difficult to see how plants can exert any major influence on pollinator flight distances by altering floral rewards. For this reason, the ability of plants to discriminate pollen genotypes after pollination^{25–27} may be of major importance if they are to achieve optimal outbreeding, assuming that pollinators transfer some minimal amount of pollen an optimal distance.

Although we suggested that an intermediate optimal outcrossing distance could be caused by intragenotypic, intergenotypic, or genotype $\times$ environmental effects, our data most strongly implicate the first effect. Between-treatment differences in seedling survivorship appeared even though we planted seeds from 1976 hand pollinations in low densities far from the maternal meadow. As they occurred in the probable absence of sibling competition and in environments not similar to those of female parents, these differences suggest that physiological compatibility between parental genotypes is important in determining offspring fitness. We are now attempting to determine the importance of intergenotypic and genotype $\times$ environmental effects in molding the breeding system of *D. nelsoni*, by measuring the influence of seed dispersal distance, sibling competition, and environmental variation on the viabilities of variously outcrossed progenies.

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Bimodal distributions of seedling weight in relation to density of *Festuca paradoxa* Desv.

THE degree to which plants growing together interfere with one another by competing for resources such as light, moisture and nutrients is difficult to determine by direct measurement of resource depletion¹. Plant ecologists have therefore looked for indicators of interference in population structure^{2,3}. One proposed measure of interference is the shift that occurs from the 'normal' distribution of seed weight to a 'log-normal' distribution of the weight of seedlings grown from those seeds in an even-aged monoculture. This shift presumably reflects the emergence of a few large and dominant individuals and the suppression of larger numbers of small individuals. Recently Ford⁴ and Newbould⁵ have proposed that distributions of plant weight in competing populations are actually bimodal, resulting from the presence of distinct populations of large individuals with positive relative growth rates and small individuals with nearly zero growth rates. Even-aged plantations of *Picea sitchensis* demonstrate such disjunct growth rates, and mortality is confined to the smaller individuals⁴. Size distributions in naturally thinning populations of *Abies balsamea* and *Prunus pensylvanica* are also bimodal⁶. We have studied the distribution of seedling weight in even-aged stands of *Festuca paradoxa*, a native North American prairie grass⁷, and report here that although the distribution is bimodal, the bimodality is more striking at lower densities, where interference is less severe. This result is the reverse of that expected if the extent of bimodality were a direct indication of the magnitude of interference. Therefore, although bimodality may be an eventual outcome of interference, it cannot be used as a measure of the strength of interaction between individuals.

At the highest density (16 seeds cm⁻²), distributions of ln-transformed seedling weight (Fig. 1) are only slightly or not at all bimodal. The distributions become increasingly bimodal as density increases. At the lowest density (1 seed cm⁻²), the distribution appears most strongly bimodal. All distributions are significantly different from the normal distribution at the 5% level (*G*-tests⁸). Only the distributions at 16 seeds cm⁻² show significant skewness (to the right⁹), but all are significantly platykurtic (flat-topped⁹). Platykurtosis is not sufficient to demonstrate bimodality, but bimodal distributions are platykurtic. There is no direct statistical measure of bimodality.

The bimodality in the weight distributions is not due to an underlying bimodality of seed weight, either for the intact diaspore or for the caryopsis without accessory tissues (Fig. 2). Neither distribution deviates significantly from the normal distribution (*G*-tests⁸), and neither is skewed or kurtotic. The

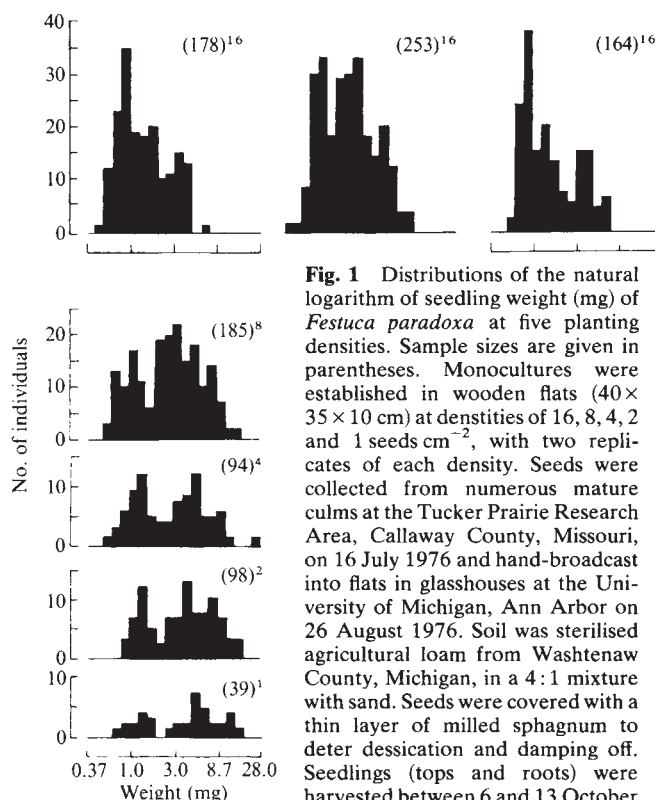


Fig. 1 Distributions of the natural logarithm of seedling weight (mg) of *Festuca paradoxa* at five planting densities. Sample sizes are given in parentheses. Monocultures were established in wooden flats (40 × 35 × 10 cm) at densities of 16, 8, 4, 2 and 1 seeds cm⁻², with two replicates of each density. Seeds were collected from numerous mature culms at the Tucker Prairie Research Area, Callaway County, Missouri, on 16 July 1976 and hand-broadcast into flats in glasshouses at the University of Michigan, Ann Arbor on 26 August 1976. Soil was sterilised agricultural loam from Washtenaw County, Michigan, in a 4:1 mixture with sand. Seeds were covered with a thin layer of milled sphagnum to deter desiccation and damping off. Seedlings (tops and roots) were harvested between 6 and 13 October 1976 in randomly-placed 5 × 5 cm plots, 3 for each density, washed to remove soil from the roots, dried at 80 °C for 24 h, and weighed on a Cahn electrobalance. For 8, 4, 2 and 1 seeds cm⁻², one-way anovas⁸ showed no differences in seedling weight among the three replicates at the 10% level. Consequently, the replicates were lumped, and one distribution is shown. At 16 seeds cm⁻², the replicates differed at the 1% level (*F*₂ = 4.87) and are shown separately. Distributions at 8, 4, 2 and 1 seeds cm⁻² are all significantly different from the distributions at 16 seeds cm⁻² (χ^2 tests⁸).

bimodality is not due to an underlying bimodality in germination time (Fig. 3). This distribution is significantly right-skewed and leptokurtic.

Mean seedling weight increases with decreasing density (Table 1), indicating that resource limitation is occurring at higher densities. The bimodal pattern emerges before density-dependent mortality. The ratio of seedlings harvested to seeds planted is not significantly different for the different densities (one-way anova with arcsine transform⁸). Mortality, if occurring, is uniform among the densities.

Interference is *a priori* more severe at higher densities. Our finding that bimodality is weaker at higher density suggests that at high density, the manifestations of interference in population structure are delayed and that the interaction between individuals is slowed. At the highest density, all individuals may

Table 1 Seedling weight of *Festuca paradoxa* at five planting densities

Planting density (seeds cm ⁻²)	Sample size	Geometric mean of seedling weight $\pm$ s.d. (mg)	Skewness <i>g</i> ₁	Kurtosis <i>g</i> ₂
16	178	1.40 $\pm$ 2.23	0.444*	-0.881*
16	164	1.58 $\pm$ 1.95	0.620*	-0.893*
16	253	1.73 $\pm$ 1.88	0.258†	-0.745*
8	189	2.78 $\pm$ 2.73	-0.086	-0.935*
4	94	2.82 $\pm$ 3.29	0.125	-0.946*
2	98	3.71 $\pm$ 3.38	-0.165	-1.133*
1	39	3.92 $\pm$ 3.82	-0.318	-1.032†

Skewness (*g*₁) and kurtosis (*g*₂) are calculated for the ln-transformed distributions shown in Fig. 1.

**P* < 0.01; †*P* < 0.05.

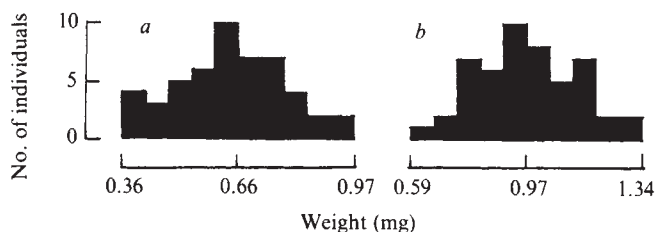


Fig. 2 Distributions of weights of excised caryopses (a) and intact diaspores (caryopses with lemmas and paleas) (b), obtained on a Cahn electrobalance. Arithmetic scale, sample size is 50 in both cases. For the intact diaspores, $\bar{x} = 0.967$ mg, $s = 0.170$, $g_1 = 0.129$ (not significantly different from zero⁹), and $g_2 = -0.576$ (NS). For the excised caryopses, $\bar{x} = 0.639$ mg, $s = 0.143$, $g_1 = 0.008$ (NS), and $g_2 = -0.502$ (NS). Neither distribution is significantly different from the normal distribution (G -test⁸).

suffer from more severe resource limitation; this idea is supported by the right skew in the data at 16 seeds cm^{-2} . The appearance of dominant individuals may be postponed. At lower densities, because resource limitation is less severe, growth is more rapid, individuals are larger, and the emergence of dominant individuals (and hence the bimodality) is more rapid.

Factors determining which individuals become dominant and which are suppressed remain somewhat obscure. Suppression is probably not due to genetic differences¹⁰. Seed size¹¹, order of emergence¹² and neighbourhood effects¹³ have been implicated in determining which individuals become dominant.

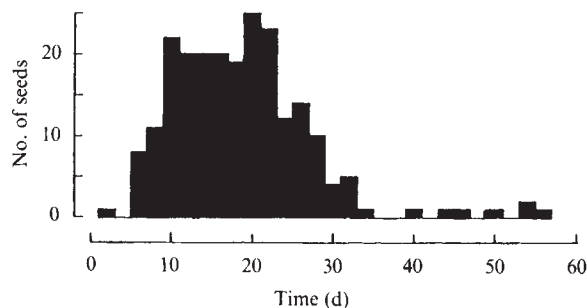


Fig. 3 Distribution of day of germination for excised caryopses of *Festuca paradoxa*. Arithmetic scale, sample size is 222. Excised caryopses between filter paper moistened with distilled water were germinated in the dark at room temperature. $\bar{x} = 18.6$ d, $s = 8.6$, $g_1 = 1.41$ ($P < 0.01$) and $g_2 = 3.78$ ($P < 0.01$).

While intrinsic factors generating bimodality cannot be exhaustively eliminated, the two most likely intrinsic sources (bimodality in seed weight or germination time) have been excluded here. The bimodality is probably a direct result of interference, although the possibility remains that severe interference prevents the expression of an intrinsic bimodality (due to other factors) at high density.

Disjunct populations with zero and positive growth rates are sufficient to generate a bimodality in distributions of $\ln$ -transformed individual weight. Bimodality appears to be an eventual structural result of interference and thus is a valuable descriptor of populational attributes. These results demonstrate the need to distinguish between populational attributes which characterise the pace and severity of interference from attributes which describe the outcome of interference.

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A method for culture of micromanipulated sheep embryos and its use to produce monozygotic twins

MICROSURGICAL techniques have proved valuable for investigations of the regulatory capacity of early mammalian embryos, including the developmental potential of single blastomeres from two-, four- and eight-celled embryos (see ref. 1 for review). However, the usefulness of radical microsurgery in experiments on early cleavage stages has been restricted largely to species in which such young embryos may be cultured *in vitro*, notably the mouse, as, in most instances, embryos less advanced than the late morula stage fail to survive *in vivo* if the zona pellucida has been removed or substantially damaged²⁻⁶. There has been no satisfactory method for culture *in vitro* of early cleavage stages in the sheep, and consequently there have been few reports of attempts to micromanipulate early sheep embryos⁷⁻⁹. I describe here a method whereby micromanipulated eggs can be protected during their early development *in vivo* and its use in an investigation of the developmental potential of single blastomeres from two-celled sheep embryos with the aim of producing monozygotic twins. The method involves coating the eggs with agar after microsurgery so as to seal defects produced in the zona pellucida.

In preliminary experiments it was established that young sheep embryos embedded in agar and transferred to rabbit oviducts¹⁰ continued to develop at a normal rate, even when the zona pellucida was extensively damaged, provided that they remained embedded in agar during the culture period. On the basis of this observation, a technique was devised which enabled the production of monozygotic twins.

Two-celled embryos were collected from super-ovulated donor ewes early on day 2 of their oestrous cycle (onset of oestrus: day 0). Embryos were collected, stored, micromanipulated and transferred in an enriched phosphate-buffered saline medium (PBS)¹¹. During storage and manipulation the embryos were held at room temperature.

Monozygotic pairs of agar-coated single blastomere eggs were produced as follows. (1) The two-celled egg was held by suction with a capillary pipette, and the zona pellucida was ripped open along the interblastomeric groove for about three-quarters of its circumference with a fine glass needle. (2) The blastomeres were sucked out of the zona with the aid of a Pasteur pipette with a tip diameter sufficiently small to detain the zona while allowing the

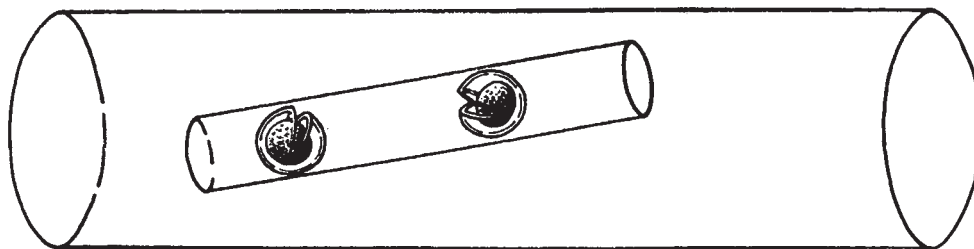


Fig. 1 Diagram of a pair of micro-manipulated single blastomere eggs and agar embedding.

blastomeres to pass through. (3) The blastomeres were separated by gentle suction with a Pasteur pipette of smaller tip bore, allowing the passage of only one, slightly compressed, blastomere at a time. (4) Single blastomeres were injected with a capillary pipette into zonae evacuated as described above and held by suction. (5) A 1.2% solution of agar (Difco) in 0.9% NaCl in distilled water was prepared. While the solution was left to cool, the eggs to be embedded were transferred from PBS to sheep serum. (6) When the agar had cooled to 38–36 °C a few ml were poured into a Petri dish, and a monozygotic pair of single blastomere eggs was immediately transferred to the agar solution with a fine-bore Pasteur pipette to be picked up a few seconds later in a small amount of agar solution. The agar containing the eggs was held in the tip of the pipette for about half a minute and then expelled as a solid cylinder into PBS. The ends were cut off the cylinder so that the eggs were contained in a segment measuring 0.15×0.5 –1.0 mm. (7) This tiny cylinder was in turn embedded by a similar procedure into a larger one, measuring 0.7×2.0 –2.5 mm (Fig. 1). Procedures (1) and (4) were carried out with the aid of a Leitz micromanipulator and De Fonbrune suction/injection devices.

One to four agar cylinders, each containing a pair of monozygotic eggs, were transferred to each oviduct of ewes on days 1 or 2 of their oestrous cycle. To facilitate collection of eggs, the oviducts of recipient ewes were ligated at the utero-tubal junction. After $3\frac{1}{2}$ to $4\frac{1}{2}$ d the eggs were flushed from the oviducts and examined. Those embryos which had reached the late

morula–early blastocyst stage were considered to have developed at a normal rate. Among the rest a distinction could be made between those which had undergone some development but were retarded and those which were completely degenerate (Fig. 2).

The results of this part of the experiment are summarised in Table 1. It is evident that very considerable losses of embryos resulted from inefficient micro- and macrosurgery on two-celled embryos and recipient ewes, respectively, but few embryos were lost in the course of the embedding procedure. Furthermore, all embryos recovered from the recipients had intact agar coatings, which made it a simple matter to recognise not only the original pairs, but also individual embryos within these pairs. The overwhelming majority of embryos recovered had also developed at an apparently normal rate. As observed in investigations of single blastomere mouse eggs^{11,16}, the time at which compaction and blastulation occurred reflected the age of the embryo rather than the number of cells it contained.

Table 1 Results of manipulation experiment

Pairs of monozygotic blastomeres	
Start of experiment (~no. of two-celled embryos)	61
After removal of zona pellucida, blastomere separation and injection into evacuated zonae	33
After embedding in agar	31
Transferred to ewes	31
Recovered from ewes	20
Stage of development of recovered single blastomere eggs	
Late morula or early blastocyst	35
Retarded	1
Degenerate	4
Monozygotic pairs in which both embryos had developed at a normal rate	16

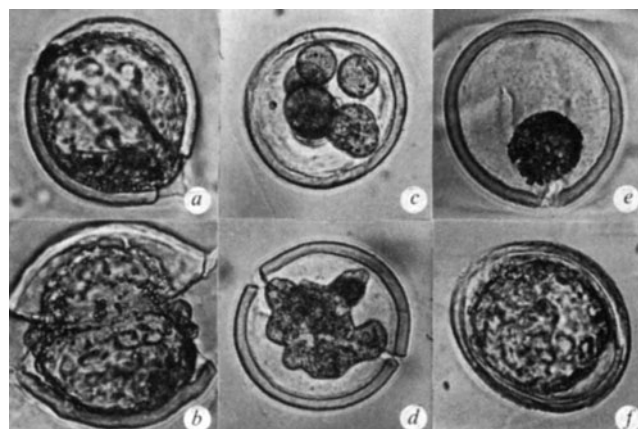


Fig. 2 Eggs from single blastomeres of two-celled sheep embryos after culture in sheep oviducts but still embedded in agar (~ $\times 400$). *a, b*, Monozygotic pair after $4\frac{1}{2}$ days' culture. Both eggs have developed at a normal rate and are early blastocysts. *c, d*, Monozygotic pair after $3\frac{1}{2}$ days' culture. *c* is retarded in its development, whereas *d* has developed at a normal rate and undergone compaction. After fixation and staining, *c* was found to contain 8 cells, *d* 16 cells, all with morphologically normal nuclei. Normally, compaction is only evident in sheep embryos containing 30 or more cells. *e, f*, Monozygotic pair after $4\frac{1}{2}$ days' culture. *e* is completely degenerate, whereas *f* is an early blastocyst. The large defects in the zonae pellucidae are visible in *a, b* and *d*. The main reason for inserting the single blastomeres into evacuated zonae was that the presence of the zona made it easier to remove the agar after culture, without damaging the embryo.

Thirty-two late morulae and early blastocysts, constituting 16 sets of monozygotic embryos, were manipulated out of the agar with a pair of 24-g hypodermic needles, and transferred to ewes on days 5, 6 or 7 of their oestrous cycle. Each ewe received one pair of monozygotic embryos. Of 16 recipients, 11 became pregnant. One aborted a single fetus on day 100. The remaining 10 went to term, producing, in all, five sets of twins (Fig. 3) and five single lambs. This result represents an embryonic survival rate 10–15% below that expected following transfer of ordinary embryos on days 5–7 in sheep^{12,13}. It is likely that damage resulting from the manipulations necessary to remove the agar before transplantation was responsible for the lower viability of embryos in the present experiments, and when the twinning rate is taken into consideration, it seems reasonable to conclude that each blastomere of a normal two-celled sheep embryo has the potential to develop into an entire lamb. In view of the work of Nicholas and Hall¹⁴, Seidel¹⁵ and Tarkowski¹⁶ on single blastomeres of two-celled embryos of rat, rabbit and mouse, this result was not surprising, although the experiments of Mullen, Whitten and Carter¹⁷, in which only one pair of monozygotic twins was obtained from the separated blastomeres of an unspecified number of two-cell embryos, suggested low viability of such 'half embryos'.

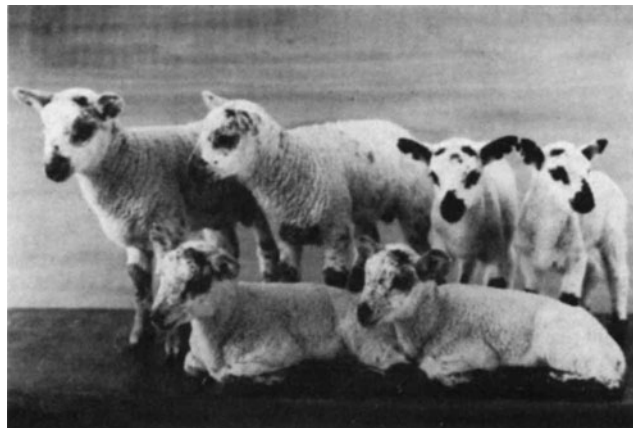


Fig. 3 Three sets of monozygotic twins produced by separation of the blastomeres of two-celled sheep embryos. Welsh Mountain cross-bred ewes, inseminated with Suffolk ram semen, were used as embryo donors. Note the general similarity in the distribution of pigmented areas, but also some minor differences between co-twins.

It may be predicted that the agar embedding method will prove useful, not only for culture *in vivo* of micromanipulated sheep eggs whether they be of the 'single blastomere' or the 'allophenic'¹⁸ variety, but also in opening the way for similar experiments in other species, such as the pig and the cow, in which early embryos cannot yet be cultured *in vitro* with any high degree of success.

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Chromosome polymorphism and sex determination in a wild population of tsetse

SEX in the Diptera is determined by a variety of genetic mechanisms ranging from the simple condition of male or female heterogamety to complex polygenic systems¹. Studies of tsetse flies have shown the males of all species examined to be heterogametic XY with a heteropycnotic Y chromosome^{2,3}. Such cytological heterogamety is found in many dipteran species but the presence of 'sex' chromosomes does not necessarily mean that they are involved in sex determination⁴. Although

there have been no studies specifically related to sex determination in *Glossina*, it has been postulated that the system is similar to that found in *Drosophila*⁵. I report here on a natural population of *Glossina palpalis palpalis* (R-D) found to contain a high incidence of aneuploids, the composition of which may be used to explain the mechanism of sex determination in the genus.

Flies were caught at five sites on tributaries of the rivers Niger and Kaduna. The sites, Akerre, Kuje, Bwari, Guni and Gimi, are located within a 100-km radius of Minna in Niger State, Nigeria. Captured females were transferred to the laboratory where they were caged individually, maintained at about 25 °C and 60–70% relative humidity and fed on rabbits. Puparia produced were collected daily and when 5 d old were used for cytogenetic analysis. Brains were dissected from the pupae, cultured for 3 h in Eagle's minimal medium containing 1 µg ml⁻¹ colchicine and fixed in methanol-acetic acid. Air-dried chromosome preparations⁶ were made from the brain tissue and stained routinely in toluidine blue.

Production of puparia by the wild-caught flies was poor, many females producing only one puparium, the maximum obtained from a single female being five. A total of 553 puparia was obtained from 249 females, an average of 2.2 puparia per female. The normal chromosome complement of *G. p. palpalis*³ is $2n = 4 + XY$, but 26 of the females in this study (10.4%) produced offspring which were aneuploid. Of these 35 abnormal pupae, 22, which were shown to be female by dissection, were found to have XXY chromosome complements, and one was found to be XXXY; seven pupae, shown to be male by dissection, were found to be XYY (Fig. 1), and a further five pupae were X0 males. All the females producing aneuploid pupae were also shown to be capable of producing normal XX female and XY male offspring. In the case of the five X0 pupae, each came from females which produced only one such aneuploid type, the

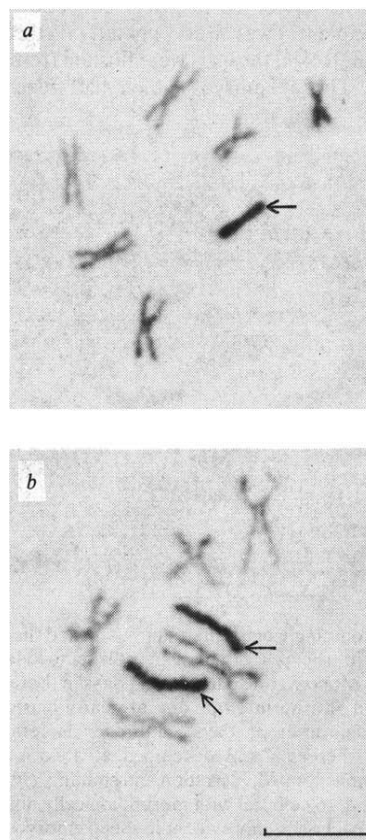


Fig. 1 Metaphase neuroblasts from 5-d-old embryos of *G. p. palpalis* showing sex chromosome aneuploidy. Arrows indicate Y chromosomes. a, Female, $2n = 4 + XXY$; b, male, $2n = 4 + XYY$. Bar = 10 µm.

rest of their offspring (average three puparia per female) being normal. These figures represent the pooled data from the five collecting sites, as no significant differences were found in the incidence of aneuploidy between sites.

The presence of an extra Y chromosome, or even two Y chromosomes, in female *G. p. palpalis*, and the absence of a Y in males were found to have no obvious phenotypic effect; thus, it may be concluded that sex in this species is determined by the number of X chromosomes present. This system of sex determination is clearly analogous to that demonstrated in *Drosophila* by Bridges' classical experiments with mutant strains⁴. It was later established that sex in *Drosophila* is determined by a system of genic balance between sex determinants on the autosomes and the X chromosome⁷. Such systems are common in insects¹ and it seems probable that the autosomes play a part in sex determination in *Glossina*; however, until useful genetic markers are available, this cannot be confirmed. The X0 male pupae found in the present study all had apparently normally developing testes, but the course of spermatogenesis could not be followed in pupae of this age. Such X0 males are completely sterile in *Drosophila* due to failures at spermiogenesis⁸, but until greater numbers of X0 males are obtained, the precise role of the Y chromosome in *Glossina* remains uncertain, although it has been suggested that it may not be involved in sperm development⁹.

The production of sex chromosome aneuploids in this population of *G. p. palpalis* can be accounted for by non-disjunction of the X chromosome during female meiosis, giving XX and 0 eggs, the fertilisation of which by normal sperm would give rise to the XXY females and X0 males found; XXY females when crossed with normal males can be expected to produce XYY males⁴. However, the frequency of spontaneous primary non-disjunction in female *D. melanogaster* is known to be very low (0.05–0.1%)¹⁰, and, although such data are not available for *Glossina*, the occurrence of 21 females (8.4%) in the present sample shown to be XXY (by producing XXY, XYY, XX and XY pupae) suggests that they are being maintained in the population as a polymorphism rather than by spontaneous non-disjunction. As *G. p. palpalis* is an important vector of sleeping sickness in West Africa, it may be of practical value to determine what selection pressures, if any, are maintaining this polymorphism in the population, as the epidemiology of the disease is intimately associated with the ecology of the fly¹¹.

If, as suggested here, sex determination in *Glossina* is controlled by a polygenic system, then it is probable that sex ratio-distorting factors are present in the tsetse genome. Such factors are commonly found in other Diptera, including mosquitoes¹² and house flies¹³, which are known to have complex sex-determining mechanisms; indeed, it has been suggested that segregation distortion may be a means whereby mutant sex determinants become established in a population¹⁴. Sex-ratio distortion has been induced in *G. morsitans* by irradiation⁵ and, in view of the present work, it would seem expedient to screen natural populations for distorting factors, as any mechanism which would increase the production of males would be most useful to tsetse control programmes which involve the release of sterile males¹⁵.

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Giardia excystation can be induced *in vitro* in acidic solutions

MANY vertebrates, including humans, are hosts for the intestinal protozoan parasite, *Giardia*. This genus has a worldwide distribution¹ and in recent years has become an increasingly important public health problem^{2–14}. Human infections may be associated with intestinal discomfort, diarrhoea, and malabsorption syndromes^{15–17}. The life cycle of *Giardia* is divided into trophozoite and cyst stages. When the cyst is ingested by the host, excystation occurs (trophozoites emerge) and the upper small intestine is colonised. Although research on *Giardia* excystation began nearly 50 yr ago^{18,19}, conditions for the induction of the process were never defined. We demonstrate here (1) *Giardia* excystation in acidic solutions, (2) the pattern of trophozoite emergence during excystation, (3) a method for routine *in vitro* induction of excystation, and (4) the establishment of *in vitro* axenic cultures from excysted trophozoites.

Cyst-bearing faeces were obtained from humans and animals, purified by a modification of previous procedures^{20,21}, and stored in water at 8 °C. We attempted to duplicate the conditions to which cysts are exposed *in vivo*. Cysts were exposed to a synthetic gastric juice, transferred into growth medium and examined microscopically. Within 5 min trophozoites were seen excysting (Fig. 1). The escape of the trophozoites resembled the extrusion of a fluid-filled balloon through a small hole in one end of the cyst.

Table 1 Excystation of *Giardia* cysts exposed to inorganic acids at pH 2.0

Acid	Mean % excystation ± s.e.m.
HCl	17.4 ± 2.2
HNO ₃	12.5 ± 3.0
HClO ₄	15.5 ± 2.0
H ₂ SO ₄	15.5 ± 2.6
H ₃ PO ₄	19.4 ± 2.8
Water (control, pH 6.8)	0.2 ± 0.2

The mean percentage of excystation is the average of 11 trials; an average total of 2,242 cysts were counted in each acid. Mean % excystation for water differs significantly from those of all acids ($P < 0.01$); mean % excystation values for the acids are not significantly different from each other ($P > 0.01$).

To identify the factor(s) responsible for the induction of excystation, complete and component-varied synthetic gastric juice at pH 1.6, HSP-3 (see Fig. 1), and water were tested for ability to induce excystation. To count cysts and assess levels of excystation, the following standard procedure was developed. One volume of purified cyst suspension was added to at least 10 volumes of excystation solution (such as synthetic gastric juice, HCl(aq), water), and the mixture incubated at 37 °C for 1 h. The suspension was then centrifuged at 600g for 5 min at room temperature, and the pellet resuspended in water and re-centrifuged. The pellet was resuspended in 0.5 ml of HSP-3 at 37 °C, and a depression slide chamber filled with the suspension, sealed with a cover glass and paraffin-Vaseline, and incubated

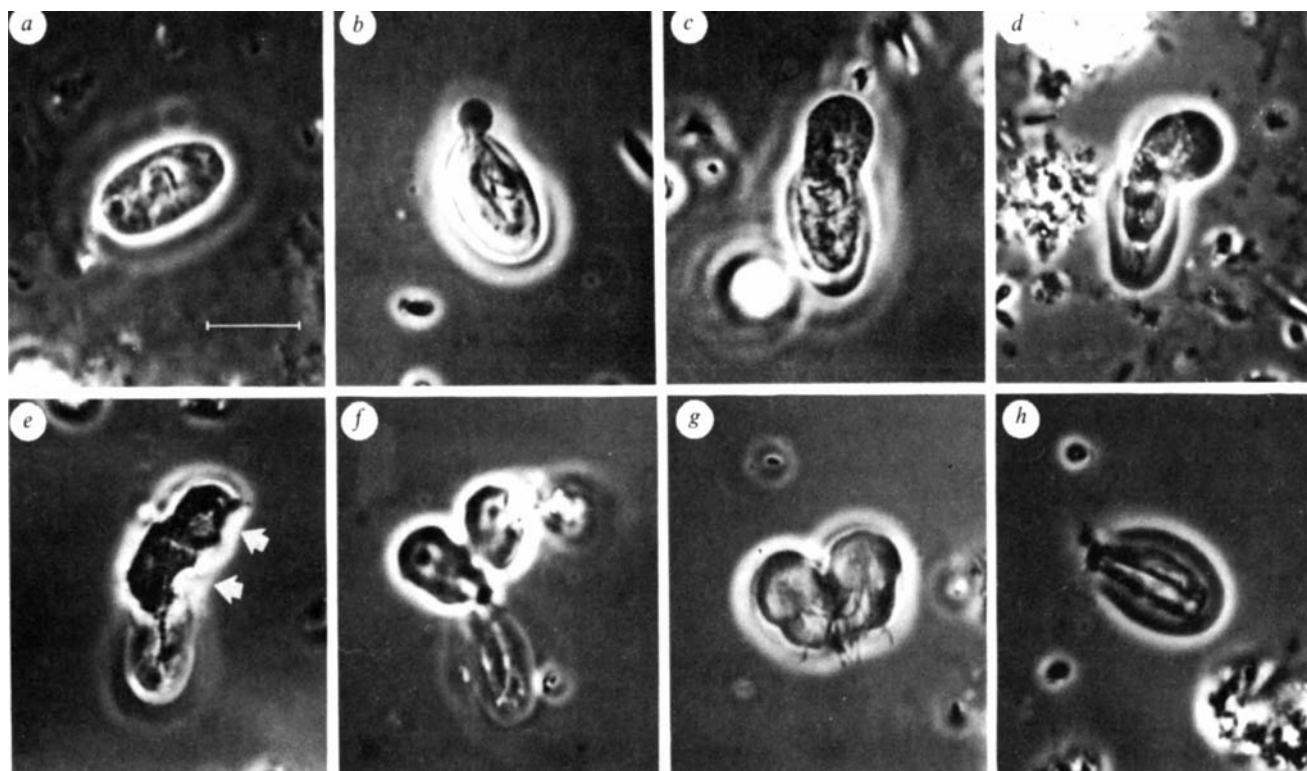


Fig. 1 Representative steps in *Giardia* excystation. *a*, Typical cyst; *b-f*, sequential emergence of trophozoite (arrows in *e* indicate ventral adhesive disks of daughter trophozoites); *g*, excystation completed, division of daughter trophozoites continuing; *h*, empty cyst. The entire process normally requires 5–30 min. Cysts were exposed to 37 °C for 2 h to a synthetic gastric juice containing the following components in aqueous solution: NaHCO₃ (25 mN), KCl (12 mN), NaCl (40 mN), CaCl₂ (12 mN), and pepsin (1,500 units ml⁻¹; Sigma); final pH 1.6 (refs 22, 23). Following exposure to the synthetic fluid, the cysts were transferred into 37 °C HSP-3, a *Giardia* growth medium derived from Meyer's HSP-1 and HSP-2 media²⁴ with the following formulation: 85 ml Hanks-phytone broth, 20 ml Seitz filter-sterilized heat-inactivated human serum, 7.5 ml NCTC-135 (Gibco), 1.5 ml 1.0 M NaHCO₃, 200,000 units potassium penicillin G, 0.01 g streptomycin sulphate and 0.01 g gentamicin sulphate; final pH 7.0. Photographs were taken under phase contrast at ×630 on a Zeiss Invertoscope D with Kodak SO-115 film. Scale bar, 10 μm.

inverted at 37 °C for 1 h. The cover glass was scanned systematically at ×300 on an inverted microscope, and cysts and trophozoites were counted. It was found that exposure of cysts to water and HSP-3 yielded negligible (<0.1%) excystation, whereas the other solutions induced 22–26% excystation. Salts and pepsin did not significantly alter the level of excystation in these solutions (Student's *t* test, *P* > 0.01), indicating that HCl alone was capable of inducing the process.

Subsequent experiments with HCl(aq) at pH values between 0.5 and 6.8 revealed peak percentages of excystation between pH 1.3 and 2.7, with a significant drop in excystation above and below peak values (*P* < 0.01), and progressively diminishing excystation as the pH approached neutrality. These results thus demonstrate a relationship between pH and levels of excystation. To determine whether the factor inducing excystation was hydrogen ion, chloride ion, or a combination of the two, several acids were adjusted to pH 2.0 and excystation attempted by the standard procedure. Each acid induced essentially identical levels of excystation (Table 1), indicating that the hydrogen ion induced excystation regardless of the counterion.

Giardia excystation therefore seems to be a pH-dependent process initiated by acidic conditions encountered during gastric passage. Using the standard excystation procedure with pH 2.0 HCl, we have induced excystation of *Giardia* from humans, monkeys, dogs, rats and mice, and have established cultures from excysted trophozoites of cysts obtained from humans and monkeys. These cultures have been maintained for up to 7 months in HSP-3, and most were cultivated axenically from the time of excystation.

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Arrested proliferation of radial glial cells during midgestation in rhesus monkey

AUTORADIOGRAPHIC analysis using ^3H -thymidine labelled DNA demonstrates that some radial glial cells in the fetal monkey cerebrum stop dividing during midgestation. After two months they re-enter the mitotic cycle and become transformed into astrocytes. In their amitotic period, radial glial cells remain stretched across the width of the rapidly expanding telencephalic wall. This is significant because elongated glial fibres may have a role in guidance of neuronal migration¹⁻³. The pause in proliferation of radial glial cells could ensure a faithful mapping of the proliferative ventricular surface upon the expanding and convoluted cerebral cortex of primates, including man³. Elongated radial glial cells span the distance between the ventricular and pial surface of the developing telencephalon (Fig. 1). Studies using Golgi silver impregnations⁴⁻⁷, electron microscopy^{1,8,9} and immunohistochemistry¹⁰⁻¹² substantiate the glial nature of this transient cell class (for review see refs 12, 13). In the monkey occipital lobe, radial glial cells are present throughout the last two-thirds of gestation^{12,14}. The process of transformation of radial glial cells into astrocytes and ependymal cells begins during the first half of gestation and is completed by the second postnatal month^{12,14}. However, during midgestation, when neuronal migration is at a peak¹⁵, many radial glial cells remain attached to the ventricular and pial surfaces (Fig. 1); they increase in length, and curve with the expansion and convolution of the cerebral wall^{3,12,14}. The present study was undertaken to determine whether such glial cells undergo cell division after attaining their radial configuration.

Brains from 53 monkey fetuses were exposed to a single pulse of ^3H -thymidine (^3H -TdR) at various stages of gestation as part of study of primate neurogenesis (Table 1)¹⁴⁻¹⁶. Some fetuses were killed 1 h after injection (column 2, Table 1) to identify the population of actively dividing cells. Others injected at comparable gestational ages were killed at 3, 7, 14 or 50–60 days later (columns 3, 4, 5, 6 respectively) to determine which cell classes continue to divide (and thereby dilute ^3H -TdR) as well as to show any changes in position of non-dividing cells after injection. Finally, some animals were killed several months after birth (column 7). Following fixation, one cerebral hemisphere from each animal was processed for autoradiography¹⁶ and the other was impregnated with the rapid Golgi method².

Two populations of cells labelled with ^3H -TdR were identified on the basis of their postmitotic behaviour. In monkeys injected at E87 (embryonic day 87) or at E90 and killed 1 h after injection, all heavily labelled cells are found in the ventricular and subventricular zones, the two proliferative areas lining the lateral ventricles (Fig. 2a). However, in specimens injected at approximately the same fetal ages, but killed 3 or 7 days after injection, many heavily labelled cells are found in the intermediate zone and in the cortical plate while a certain number of heavily labelled cells remain in the proliferative zones. In animals killed 14 days after injections at E85 or E89, most of the migrating labelled cells have already reached their position in the superficial layers of the cortex¹⁴; a certain number of labelled cells are, however, still found near the ventricle at this time. In two additional fetuses injected at E85 and E90, but killed after approximately 2 months (E139 and E140, respectively), all migrating cells generated at E85 or E90 have reached their final destinations as neither the intermediate zone nor the deep cortical layers contain labelled cells. The shift in location of heavily labelled cells reflects the well documented pattern of neuronal migration to the neocortex¹⁵. Again, in these same cases, however, a substantial number of heavily labelled cells persist in the outer part of the subventricular zone and at the ventricular surface. The number of these cells is particularly high

in areas underlying large cerebral convolutions where they form wedge-shaped aggregates (Fig. 2b).

These observations indicate that during the 2-month interval between ^3H -TdR injection and death, a certain number of cells in the subventricular zone do not divide. These non-dividing cells are unlikely to be neurones which in this region complete their proliferation by E100 and reach their final destination before E120 (refs 14, 15). The large pale nuclei of the labelled cells (Fig., 2c) have characteristics ascribed to radial glial cells by light and electron microscopy¹. Furthermore, their location and pattern of distribution in the subventricular zone correspond to the dense concentration of radial glial somas revealed by the Golgi silver impregnation method in the opposite cerebral hemispheres of the same E138–E140 fetuses (Fig. 1b).

In specimens injected with ^3H -TdR at E140 and killed 1 h later, a substantial number of large pale nuclei of radial glial cells

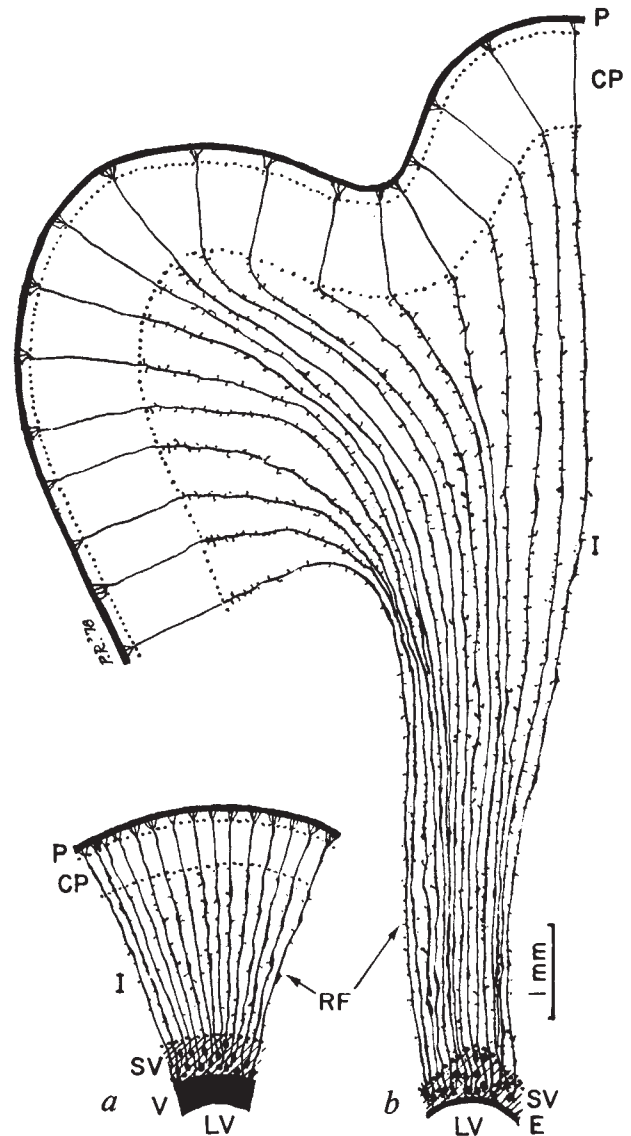


Fig. 1 Semi-schematic drawing of the distribution of radial glial cells in the telencephalon of two monkey fetuses stained with Golgi method. *a*, Dorsal portion of the occipital lobe of 81-d-old fetus (E81); *b*, section from the corresponding region from a fetus at E138. Due to the increased thickness of the cerebral wall and expansion of cortical plate during the intervening period, radial glial fibres that traverse the cerebral wall from the subventricular zone near the lateral ventricle to the pial surface become several times longer and considerably curved. Abbreviations: CP, cortical plate; E, ependyma; I, intermediate zone; LV, lateral ventricle; P, pial membrane, RF, radial fibre; SV, subventricular zone; V, ventricular zone. For more details see refs 1, 12 and 14.

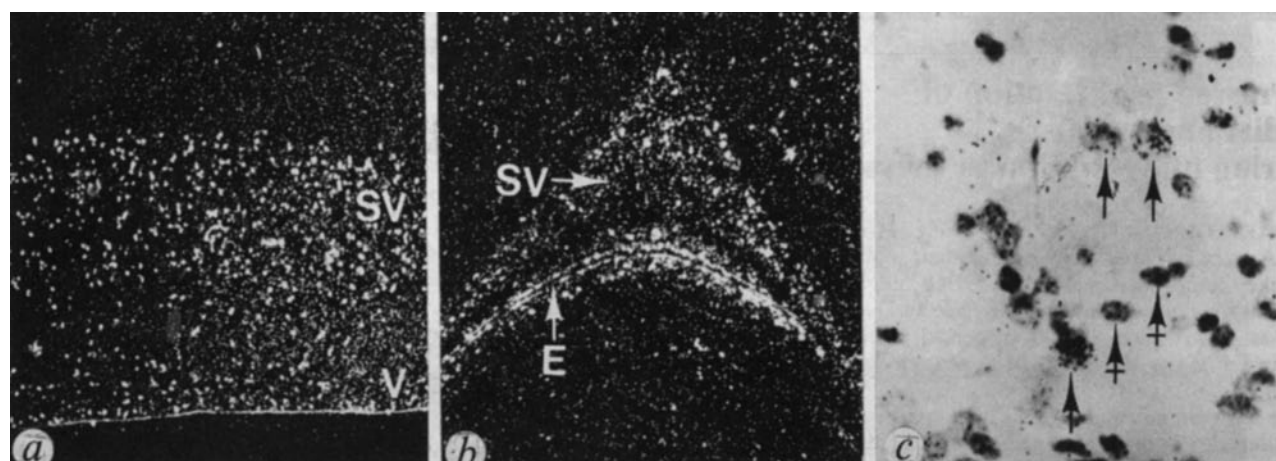


Fig. 2 *a*, Low magnification dark-field illumination photograph of the ventricular (V) and subventricular (SV) zones of the occipital lobe of a monkey fetus injected with ^3H -thymidine at embryonic day 90 and killed 1 h later. The confluence of silver grains over radioactive nuclei is such that labelled cells at this magnification appear as solid bright dots. Labelled cells are present in both proliferative zones. *b*, Similar photograph of the corresponding area in monkey fetus injected at E90 but killed at E140. By this fetal age the ventricular zone is exhausted and is replaced with the endependymal layer (E). Although during the 50-day period between injection and death most labelled cells have migrated to the cortex, many heavily labelled cells are still present in the subventricular zone. In the areas subjacent to large convolutions like the one selected here, aggregates of labelled cells form wedges that correspond to the accumulation of radial glial cell somas in Golgi impregnation (for example, see Fig. 1*b*). *c*, Bright-field photograph of large, pale, labelled nuclei (arrows) and unlabelled dark nuclei (crossed arrows) in the subventricular zone of a monkey fetus injected at E85 and killed at E139. The dark unlabelled nuclei apparently represent regular proliferating population of subventricular cells which incorporate ^3H -thymidine throughout gestation, but never retain label over long periods.

are labelled in the subventricular zone. However, in monkeys injected at either E90 or E140 but killed postnatally, no labelled cells can be detected in this general region of the occipital lobe except for an occasional endependymal cell. This finding suggests that a certain number of radial glial cells, which have not divided for almost 2 months, re-entered the mitotic cycle around E140 and thereafter divided a sufficient number of times to dilute label beyond detection by autoradiographic method.

Not all radial glial cells in the monkey pass through this amitotic period. It seems that the majority of cells that enter this dormant phase are generated during midgestation. This explains why in specimens killed 14 days after a ^3H -TdR injection given earlier or later than the midgestational period (Table 1) only occasional cells in the subventricular zone remain heavily labelled. Evidently, relatively few radial glial cells generated at those fetal ages become non-dividing. Why some radial glial cells enter a mitotically dormant stage, and what induces them to

re-enter the mitotic cycle remain to be determined. It is noteworthy that radial glial cells in the amphibian optic tectum can be induced to re-enter their mitotic cycles by transection of the optic nerve¹⁷. Thus, it is possible that the interaction of the radial fibre with the neuropil below the pial surface may mediate the proliferative activity of glial cell somas situated near the ventricular surface.

The non-dividing cell population in the primate subventricular zone is formed during the period of rapid expansion of the cerebral wall and formation of the cerebral convolutions. The 2-month dormant period during midgestation would explain how some radial glial cells maintain stable positions at both surfaces of the cerebral wall during this period (Fig. 1*a, b*). Their amitotic state may be correlated with their proposed function as guides for migration of late-generated neurones¹ and may assure columnar grouping in the cortex of neurones generated at common sites in the proliferative zones³.

Table 1 Specimens used for the determination of proliferation of glial cells at various stages of development

(1) Age-matched grouping	Survival after ^3H -thymidine injection					
	(2) 1 h	(3) 3 days	(4) 7 days	(5) 14 days	(6) 1-2 months	(7) Killed after birth
E40-43	E41-E41.1	E41-E44	E40-E47	E40-E54	E41-E83	E40-P62
E44-48	E45-E45.1	E45-E48	E46-E53	E45-E59	E45-E78	E45-P58
E49-53	E50-E50.1	—	—	E50-E64	—	E50-P61
E54-60	E58-E58.1	—	—	E56-E70	—	E56-P66
E61-67	E62-E62.1	—	—	E62-E76	—	E62-P50
E68-74	E69-E69.1	—	—	E70-E84	—	E70-P98
E75-81	E75-E75.1	—	—	E77-E91	—	E80-P48
E82-88	E87-E87.1	E82-E85	E85-E92	E85-E99	E85-E139	E85-P94
E88-97	E90-E90.1	E92-E95	E90-E97	E90-E104	E90-E140	E90-P63
E98-108	E105-E105.1	—	—	E100-E114	—	E102-P65
E109-117	E110-E110.1	—	—	E110-E124	—	E110-P62
E118-130	E120-E120.1	—	—	E120-E134	—	E120-P113
E131-149	E140-E140.1	E135-E138	E142-E149	E135-E149	—	E140-P57

Column¹ (age-matched groupings) refers to the time period during which a single intravenous injection of ^3H -thymidine (^3H -TdR) was administered to the pregnant monkey. Gestation in rhesus monkey lasts about 165 days. Animals are killed at various intervals after injection, as indicated by the column headings⁽²⁻⁷⁾. All specimens in any one horizontal row make up one age-matched grouping. E = Embryonic day, P = postnatal day. Each hyphenated set of ages in columns 2-7 represents a single monkey, indicating first the age at time of ^3H -TdR injection and then the age at the time of death. The numeral 1 in the 2nd column (for example, E58-58.1) indicates survival time of 1 h following injection at a given embryonic day.

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Production of an H-2-related suppressor factor by a hybrid T-cell line

CELL FUSION has been used to study problems in genetics, neoplasia and virology¹. Its application to immunology was initiated by the experiments of Köhler and Milstein², in which it was shown that a myeloma cell (a neoplastic B cell) could be fused with a normal antibody-producing B cell, and that the hybrids continued to secrete the specific antibody of the latter and grew as permanent lines. This type of fusion has since been used to produce homogeneous antibodies to a variety of antigens^{3,4}. The same principle can also be applied to T cells, with the aim of producing T-hybrid lines with characteristic T-cell functions, such as cytotoxicity or the ability to help or suppress the responses of other lymphocytes. Recently, fusion of T cells with T-lymphoma cells has been described, leading to the establishment of lines carrying markers characteristic of the normal T-cell partner^{5–7}, and some lines with T-cell activities have been reported^{8,9}. We describe here the production of a hybrid line which exhibits a characteristic T-cell function, namely, specific suppression of the antibody response. The hybrid cells produce a suppressor molecule or 'factor' which binds to an antigen, sheep red blood cells (SRBC), and specifically suppresses the antibody response to this antigen *in vitro*. The factor is non-immunoglobulin, but carries determinants of the H-2 complex.

Hybridisation was carried out using the thymoma line BW 5147 (derived by Dr R. Hyman), which lacks the enzyme hypoxanthine guanine phosphoribosyl transferase (HGPRT) and consequently can be killed by medium containing HAT (hypoxanthine, aminopterin, thymidine)¹⁰; 5×10^7 thymoma cells were fused with 2×10^8 spleen cells of C57BL/10ScSn mice which had been immunised 5 d previously by injection of 0.1 ml 10% SRBC. Polyethylene glycol was used as fusing agent, after the method of Pontecorvo¹¹ as modified by Goldsby *et al.*⁵. After fusion, cells were seeded at about 10^6 per well in 0.2-ml volumes in 96-well culture plates (Falcon) and cultured in

selective medium, that is, RPMI 1640 supplemented with 15% fetal calf serum and HAT. After 3 weeks, 58 of 214 wells seeded showed positive growth, indicating that they contained hybrid (HGPRT-positive) cells; their contents were transferred in 1-ml volumes to 24-well culture plates (Linbro) for continued growth. Further cloning of these cells was not carried out. Karyotypic analysis of several lines showed that the average chromosome number was between the normal mouse diploid (40) and tetraploid, and was in all cases higher than that for BW 5147, which has a chromosome number close to 40.

Supernatants of growing hybrid lines were tested for their ability to suppress primary antibody responses *in vitro* of either mouse spleen cells or mouse thoracic duct lymphocytes (TDL) against SRBC and other antigens. Although most supernatants, including that of BW 5147 cells, failed to influence the antibody response, a striking suppressive effect was obtained with that of a line designated A1. Table 1 shows that the A1 supernatant produced suppression of about 90% of the primary (IgM) response of spleen cells to SRBC but did not suppress the response to donkey red blood cells (DRBC). Moreover, the suppressive effect could be abolished by absorption of the A1 supernatant with SRBC, not with red cells of other species.

Using mouse TDL in a microculture system¹², the supernatant produced complete suppression of the response to SRBC and could be titrated to an endpoint of 50% suppression at a dilution

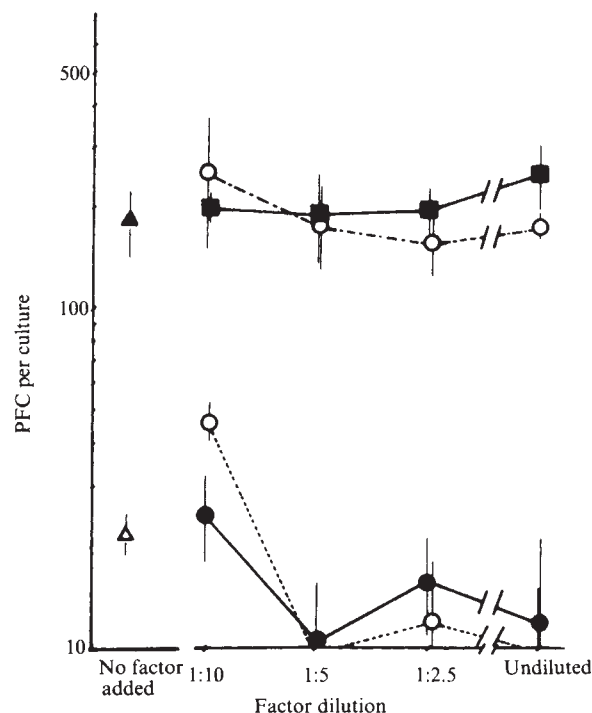


Fig. 1 Suppression of the antibody response to SRBC *in vitro* by supernatant factor of the A1 hybrid line. The figure shows the primary (IgM) antibody response to SRBC of mouse thoracic duct lymphocytes (TDL) in the presence of supernatant (factor) of A1 (●—●), A1 supernatant after absorption by SRBC (○- - -○) or by DRBC (○...○), or supernatant of another line, A3, as control (■—■). The level of response in the absence of added supernatant is shown (▲), as is the background in the absence of SRBC (△). The method will be described in detail elsewhere¹². In brief, 0.5×10^6 TDL of C57BL/10ScSn mice (an overnight collection of mouse thoracic duct cannulated according to ref. 20) were incubated with 1×10^6 SRBC and 25 μ l of the factor to be tested (or a dilution thereof) in a total culture volume of 225 μ l, in round-bottomed microtitre trays (Linbro IS-MRC-96TC) under 5% CO_2 /air at 37 °C. The culture medium was RPMI 1640 supplemented with 10% fetal calf serum, 2 mM glutamine, 100 U ml^{-1} penicillin-streptomycin and 1×10^{-5} M 2-ME. After 4 d, cells in each well were collected and direct plaque-forming cell (PFC) numbers determined¹⁹. Each point is the geometric mean of triplicate cultures $\pm$ s.d.

Table 1 Suppression of the primary antibody response *in vitro* by supernatant of the T-hybrid line A1

Supernatant	Primary PFC response to:		DRBC	% Suppression
	SRBC	% Suppression		
None	3.29(1960) ± 0.05		3.06(1160) ± 0.07	
BW 5147	3.25(1760) ± 0.03	10	3.09(1240) ± 0.04	0
Line A3	3.31(2040) ± 0.06	0	3.01(1030) ± 0.05	11
Line A1	2.24(174) ± 0.13	91	2.99(986) ± 0.05	15
A1 after absorption by:				
SRBC	3.24(1720) ± 0.07	12	ND	
DRBC	2.45(280) ± 0.15	86	ND	
Ox RBC	2.62(420) ± 0.10	79	ND	
anti-mouse antiserum	2.53(338) ± 0.20	83	ND	
anti-IgM	2.19(158) ± 0.19	92	ND	
anti-IgG _{2a}	2.48(300) ± 0.13	85	ND	
anti-IgG _{2b}	2.56(360) ± 0.22	82	ND	
anti-IgA	2.31(204) ± 0.24	89	ND	
anti-H-2 ^k	2.28(193) ± 0.23	90	ND	
anti-H-2 ^b	3.33(2130) ± 0.03	0	ND	

Results are primary IgM plaque-forming cell (PFC) responses of spleen cell cultures. Spleen cells of non-immunised C57BL/10ScSn mice were suspended at a density of 6×10^6 per ml and incubated with $50 \mu\text{l}$ 1% red cells in 1-ml volumes in culture tubes (Falcon) for 5 d, as described by Fridman and Gisler¹⁸. The culture medium was RPMI 1640 with addition of 2 mM glutamine, 1 mM pyruvate, 100 U ml^{-1} penicillin-streptomycin, 10% fetal calf serum, 1% horse serum, 5×10^{-5} M 2-mercaptoethanol (ME), and tubes were incubated under 5% CO₂/95% air. At the start, $100 \mu\text{l}$ of hybrid supernatant, taken after 48 h incubation of 5×10^5 hybrid cells in fresh medium (RPMI 1640, 15% fetal calf serum, glutamine, pyruvate, antibiotics as above) was added. Direct PFC were enumerated on day 5 in agar-free chambers¹⁹. Results as PFC per 10^6 viable cells, expressed as log₁₀ geometric means of quadruplicate cultures (anti-log in parentheses) ± standard deviation. For absorption by red cells, 1 ml A1 supernatant was mixed with 0.1 ml 50% red cells for 1 h at room temperature and then centrifuged. For other absorptions, supernatant was passed over immunoadsorbents prepared by conjugation of antisera to CNBr-activated Sepharose (Pharmacia). Anti-mouse antiserum was raised in sheep against whole mouse serum; the specific anti-immunoglobulin reagents were also sheep anti-mouse and were anti-MOPC 104E(IgM, λ), anti-Adj PC5 (IgG_{2a}, κ), anti-MOPC 141 (IgG_{2b}, κ) and anti-MOPC 315 (IgA, λ). Anti-H-2^k was C57BL/10 anti-C3H; anti-H-2^b was C3H anti-C3H.B10. Line A3 was a non-suppressor line from the same hybridisation, as negative control. DRBC, donkey red blood cells. ND, not done. Background (antigen omitted from culture): 1.89(79) ± 0.30 (SRBC), 1.60(40) ± 0.33 (DRBC).

Table 2 Suppression by T-hybrid line A1 supernatant of the primary response of C57BL/10 thoracic duct lymphocytes to SRBC and TNP₁₀-SRBC *in vitro*

Antigen	Hybrid supernatant	Undiluted	Dilution of supernatant			
			1 : 2.5	1 : 5	1 : 10	1 : 20
SRBC	Line A3	2.32(209) ± 0.04	ND	ND	ND	ND
	Line A1	0.30(2) ± 0.32	0.38(2) ± 0.29	1.52(33) ± 0.19	1.88(76) ± 0.13	2.27(186) ± 0.15
TNP ₁₀ -SRBC*	Medium control		2.36(230) ± 0.04			
	Background		1.13(13) ± 0.34			
	Line A3	2.15(140) ± 0.20	ND	ND	ND	ND
	Line A1	0.50(3) ± 0.33	0.30(2) ± 0.38	1.13(13) ± 0.35	1.52(33) ± 0.18	2.12(131) ± 0.08
	Medium control		2.09(123) ± 0.04			
	Background		0.38(2) ± 0.22			

Cultures of TDL were set up as described in the legend to Fig. 1, the initial cell number being 0.5×10^6 per culture. Either SRBC or trinitrophenylated SRBC (TNP₁₀-SRBC, ref. 21) was added as antigen (1×10^6 per culture), together with $25 \mu\text{l}$ of hybrid supernatant or a dilution thereof. After 4 d, direct PFC were measured against SRBC or TNP₁₀-DRBC*. Results as log₁₀ geometric means PFC per culture (anti-log in parentheses) ± standard deviation. Line A3 was a non-suppressor line used as negative control. As medium control, normal medium was used instead of hybrid supernatant, and for background values, antigen was omitted from the cultures. ND, not done.

Table 3 Binding of anti-immunoglobulin and anti-H-2 antibodies to SRBC treated with supernatant of T-hybrid lines

Hybrid supernatant	Binding (c.p.m.)			
	Anti-Fab	Anti- μ	Anti-H-2 ^b	Anti-H-2 ^k
None	73 ± 14	93 ± 20	86 ± 16	107 ± 17
Line A3	46 ± 9	81 ± 19	114 ± 21	88 ± 23
Line A1	58 ± 10	70 ± 12	701 ± 66	65 ± 11
Positive controls (anti-SRBC antiserum)		anti-Fab: 4,330 ± 263		
		anti- μ : 3,720 ± 186		
c.p.m. of ¹²⁵ I-reagents		anti-Fab: 15,500 ± 294		
		anti- μ : 14,200 ± 318		
Machine background		35 ± 4		

$100 \mu\text{l}$ of each supernatant was reacted with $25 \mu\text{l}$ 10% SRBC; cells were washed 3 times, then incubated with $25 \mu\text{l}$ of ¹²⁵I-labelled sheep anti-mouse Fab or sheep anti-mouse μ , or with $25 \mu\text{l}$ undiluted anti-H-2 antisera followed, after washing, by the ¹²⁵I-anti-Fab reagent. As positive controls SRBC were treated with mouse anti-SRBC antiserum followed after washing by the labelled anti-Fab or anti- μ . After further washing, the c.p.m. bound to red cells were counted. Anti-mouse Fab was raised in sheep against the Fab fragment of Adj PC5 (IgG_{2a}, κ); anti- μ was sheep anti-MOPC 104E (IgM, λ) absorbed with λ light chains; anti-H-2 antisera were as in Table 1. Results as means of duplicate tests ± standard deviation. Line A3 was a negative control supernatant; supernatants of 36 other lines also gave negative results.

of 1:20 (a final dilution in the culture of 1:180), as shown in Figs 1 and 2. Here too, absorption of the supernatant with SRBC removed its suppressive activity. However, a nonspecific suppression of TDL was evident at high concentrations of supernatant, when it was possible to suppress the responses to trinitrophenylated (TNP)-DRBC or TNP-ovalbumin as well as to SRBC (Fig. 2). Nevertheless, specificity for SRBC appeared as the supernatant was diluted beyond 1:2.5. Although the ability of the A1 supernatant to suppress the anti-TNP response to TNP-DRBC was clearly weak (Fig. 2), it suppressed the response to TNP in the form of TNP-SRBC as effectively as the response to SRBC itself (Table 2). Thus, suppression extended to haptens attached to SRBC and was evidently carrier specific. To date, we have found the supernatant to be equally effective on C57BL/10 (H-2^b), CBA (H-2^k) and BALB/c (H-2^d) TDL. The production by the A1 line of a suppressor factor with specificity for SRBC proved to be a stable characteristic of the line and could be demonstrated with supernatants obtained after passing the cells for 7 months in culture.

The nature of the A1 suppressor molecule was explored by passing the supernatant over immunoabsorbents to which anti-mouse immunoglobulin (mIg) or mouse alloantisera had been chemically coupled. Table 1 shows that anti-mIg adsorbents were unable to remove the suppressive activity. In contrast, this activity was lost if the A1 supernatant was passed over certain anti-H-2 adsorbents; specifically, immobilised antisera against the haplotype of the primed spleen cells used in the fusion (H-2^b) were able to remove the A1 molecule, whereas those reacting with the haplotype of the thymoma (H-2^k) could not. Similarly, it was shown that the molecules in the A1 supernatant binding specifically to SRBC carried H-2 determinants and were non-Ig. Table 3 shows that SRBC treated with the A1 supernatant failed to bind radiolabelled anti-mIg reagents (anti-Fab, anti- μ), but did bind anti-H-2^b antisera (detected by subsequent binding of radiolabelled anti-mIg). In other experiments this reaction was shown to be specific for SRBC. These results indicate that the A1 suppressor factor is an SRBC-binding molecule which is non-Ig and carries determinants coded in some portion of the H-2 complex. The structure of this molecule was explored further after radiolabelling, as described in the accompanying paper¹³.

Surface antigens of the A1 line were detected using antisera against H-2, mIg and Thy-1. A1 cells expressed the H-2 antigens of both BW 5147 and C57BL/10 parent cells (H-2^k and H-2^b) and both Thy-1 alleles (Thy-1.1 and Thy-1.2, respectively). A1 and all other T-hybrid lines failed to react with a variety of anti-mIg reagents, including radiolabelled sheep anti-mouse Fab and anti- μ , and fluoresceinated anti-whole mouse Ig. Similar results have been found by others^{6,7} and suggest either that BW 5147 does not hybridise with B cells or that Ig synthesis is always repressed by BW 5147 genes. The A1 line formed rosettes with SRBC, indicating the presence of receptors for SRBC at the cell surface. However, we have found formation of rosettes with red cells of certain species, particularly bovine and sometimes sheep, to be a property of many T-hybrid lines, but not of BW 5147 itself. It is also seen with lines formed by fusion of myelomas with B cells, and in the majority of cases it is apparent that rosette formation is unrelated to the antigen specificity of the lines; its significance is not yet clear. Although many T-hybrid lines bind heterologous red cells, only A1 has so far been found to release soluble material reacting with red cells. (The phenomenon of rosette formation by hybrid lines will be published elsewhere¹⁴.)

The results described here show that it is possible to produce by fusion a hybrid line which retains a functional characteristic of a normal T-cell population after antigen stimulation. This and similar lines^{8,9} promise to be of considerable value in studying the properties of the molecules produced by normal T cells, including receptors for antigen. From the characteristics of the A1 line, we suggest that the normal parent cell in the fusion may have been an SRBC-specific suppressor T cell. Thus, the suppressor molecule it releases resembles the antigen-specific

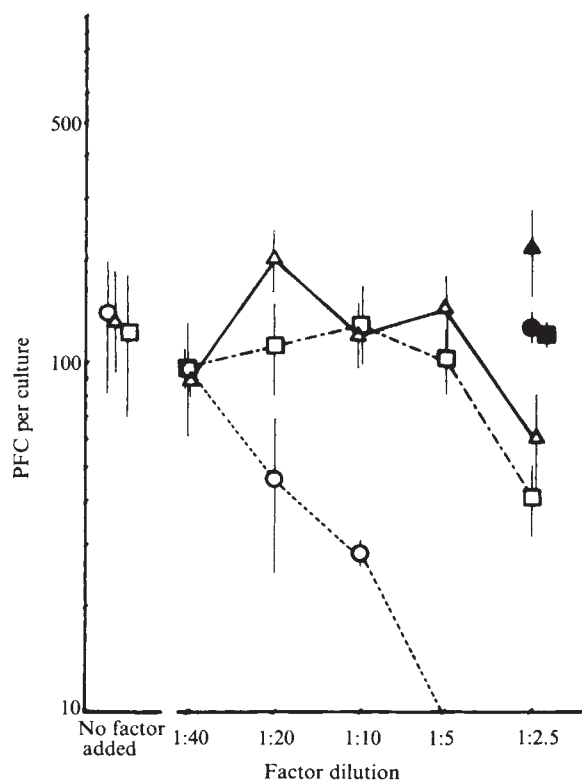


Fig. 2 Specificity of suppression of the antibody response by A1 supernatant factor. Culture conditions were as described in Fig. 1, using in this experiment TDL of CBA mice. Antigens added to the cultures were 1×10^6 SRBC (○), 1×10^6 TNP₁₀-DRBC (△) or 0.125 μ g TNP₅-ovalbumin (□), together with 25 μ l of A1 factor or a dilution thereof (trinitrophenylation of DRBC was according to ref. 21). PFC were determined against SRBC or TNP₁₀-SRBC²¹ after 4 d. Controls are shown in which supernatant of the A3 line was used instead of A1 (closed symbols) or in which no supernatants were added.

factors which have been found in cultures or extracts of antigen-stimulated T cells and which can replace T cells in regulation of the antibody response^{15,16}. Although the A1 line has not yet been cloned, it is likely that the factor it produces is homogeneous and recognises a single type of determinant on SRBC. Nevertheless, it is able to suppress the anti-SRBC response completely and can also inhibit the response to a hapten coupled to SRBC. Thus, it seems unlikely that its suppressive action is due solely to blocking of antigenic determinants on the red cell surface. A direct effect on lymphocytes is indicated by the apparently nonspecific suppression which A1 supernatants produce when at sufficiently high concentration (Fig. 2) and by more recent observations we have made which indicate that the A1 factor is absorbed by B cells in the absence of SRBC¹⁷. Our hypothesis is that the factor reacts with both B lymphocytes and SRBC and that its greater effectiveness against the latter than against other antigens is due to its binding site for SRBC, which causes it to be concentrated at the surface of SRBC-binding B cells.

An important point of similarity with other specific T-cell factors is that although the A1 factor binds specifically to SRBC, it lacks Ig determinants and carries antigens coded by the H-2 complex^{15,16}. Until now the main drawback to determination of the structure of antigen-specific T-cell factors has been the very small amount of material available, a problem which is overcome by the release of factors by permanent hybrid lines. In the accompanying report, some molecular characteristics of the suppressor factor produced by the A1 line are described.

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Structure of an antigen-specific suppressor factor produced by a hybrid T-cell line

THE preceding report¹ described the production by the hybrid T-cell line A1 of a suppressor factor with specificity for sheep red blood cells (SRBC). The factor, which binds specifically to SRBC and suppresses the antibody response to this antigen in culture, does not react with anti-mouse immunoglobulin (mIg) reagents, but is removed by mouse alloantisera (anti-H-2). In these respects it resembles the antigen-specific T-cell factors which have been obtained from T cells after stimulation by antigen and which play an important part in regulation of the antibody response^{2,3}. We report here our study of the structure of the A1 suppressor molecule by incorporation of ³H-leucine, followed by SDS-polyacrylamide-gel electrophoresis (SDS-PAGE). The molecule seems to be composed of two types of polypeptide chain in noncovalent association, a large chain of molecular weight 85,000 and a small chain of 25,000. The MW of the native molecule is about 200,000. The isolated large chain binds to SRBC. The H-2 determinants are apparently present on the small chain.

The origin of the A1 line, as a result of fusion between the AKR (H-2^k) thymoma line BW 5147 and spleen cells of a C57BL/10ScSn (H-2^b) mouse injected with SRBC, and the properties of the supernatant of the line, have been described in the preceding report¹. The molecular weight of the A1 suppressor factor was determined by gel filtration on an ultrogel AcA 34 column (LKB), eluted fractions being tested for ability to suppress the primary response of murine thoracic duct lymphocytes against SRBC, as previously described¹. The maximum suppressor activity emerged in the same fractions as a 7S IgM marker, indicating an apparent MW of about 200,000. No activity was found in fractions corresponding to MWs of less than 150,000 (Table 1).

Internal labelling of the A1 factor was achieved by allowing the cells to incorporate ³H-leucine. Cells at a density of 2×10^6

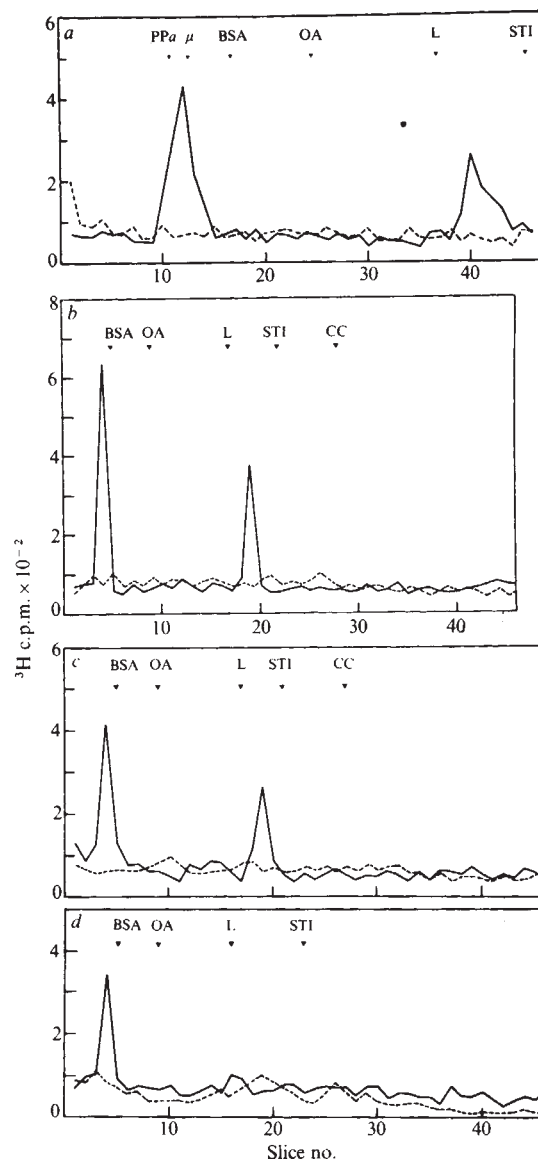


Fig. 1 SDS-PAGE profiles of SRBC-binding factor produced by the A1 hybrid line. *a*, Electrophoresis on 10% polyacrylamide gel of SRBC treated with ³H-leucine-labelled supernatant of A1 (—) or BW 5147 (---). 100 μl of the labelled supernatant was incubated with 10 μl packed SRBC for 1 h at room temperature. The red cells were then washed 3 times, dissolved in SDS sample buffer in the presence of 2-ME and phenylmethanesulphonyl fluoride, heated at 100 °C for 2 min and run according to the discontinuous system of Laemmli⁴. Markers were phosphorylase *a* (PPa, MW 92,000), heavy (μ) and light (L) chains of MOPC 104E, bovine serum albumin (BSA, MW 68,000), ovalbumin (OA, MW 45,000), and soybean trypsin inhibitor (STI, MW 21,000). *b*, Electrophoresis on 15% polyacrylamide gel of SRBC treated with ³H-leucine-labelled supernatant of A1 (—) or BW 5147 (---). Red cells prepared as in *a*. Markers as in *a* with addition of cytochrome *c* (CC, MW 12,500). *c*, Electrophoresis on 15% polyacrylamide gel of SRBC treated with ³H-leucine-labelled supernatant of A1 after precipitation by anti-H-2^k (—) or anti-H-2^b (---) antisera and sheep anti-mouse whole serum. 100 μl of the labelled supernatant was mixed with 20 μl undiluted anti-H-2^k (C57BL/10 anti-C3H) or anti-H-2^b (C3H anti-C3H.B10) antiserum, followed after 1 h by 250 μl sheep anti-mouse whole serum. After removal of the precipitate 10 μl packed SRBC was added to the supernatant and after 1 h at room temperature the red cells were washed and run on SDS-PAGE as in *b*. Markers as above. *d*, Electrophoresis on 15% polyacrylamide gel of fixed SRBC treated with NP40 extracts of ³H-leucine-labelled A1 cells (—) or BW 5147 cells (---). 10 μl packed glutaraldehyde-fixed SRBC was added to 30 μl NP40 extract (see text); after 1 h at room temperature the red cells were washed and run on SDS-PAGE as in *b*. Markers as above.

Table 1 Determination of molecular weight of A1 suppressor factor by gel filtration

Fraction no.	PFC	%Suppression
21	2.21(162)±0.08	0
23	2.29(195)±0.10	0
25	1.70(50)±0.18	71
27	1.02(10)±0.28	99
29	1.08(12)±0.15	97
31	1.30(20)±0.25	92
33	2.06(115)±0.11	25
35	2.20(160)±0.18	0
37	2.14(138)±0.12	9
39-61	No suppression in any fraction	
Medium control	2.18(151)±0.10	
Background	0.90(8)±0.25	

5 ml of A1 supernatant was concentrated to 0.8 ml, centrifuged to remove aggregates, and the sample applied to an ultrogel AcA 34 column (dimensions 93 cm × 2 cm²). The column was run in phosphate-buffered saline. 3-ml fractions were collected and alternate fractions from no. 21 onwards were tested for ability to suppress the primary response to SRBC *in vitro* of mouse (C57BL/10) thoracic duct lymphocytes, as previously described¹. Markers for MW were run separately and were shown reproducibly to emerge maximally in the following fractions: 23, blue dextran (exclusion volume); 27, 7S IgM (partial reduction product of MOPC 104E, MW 216,000); 37, μ chain of MOPC 104E (MW 80,000); 41, bovine serum albumin (MW 68,000); 44, ovalbumin (MW 44,000); 47, light (λ) chain of MOPC 104E (MW 28,000); 61, phenol red. PFC results as log₁₀ PFC per culture (anti-log in parentheses) ± standard deviation. For medium control, normal medium was used instead of fraction, and for background values, SRBC were omitted from culture.

per ml were incubated for 20 h in leucine-free RPMI 1640 medium (free of fetal calf serum), supplemented with 100 μ Ci per ml of ³H-leucine (Amersham). The labelled supernatant was allowed to react with red cells of various species and was found to bind optimally to SRBC, with some detectable binding to rabbit red cells, but none to mouse, ox or donkey (Table 2). This binding did not occur if the labelled supernatant was first treated with anti-H-2^b antiserum, followed by precipitation with sheep anti-mouse whole serum; in contrast, anti-H-2^k antiserum failed to remove the binding activity in this test, as shown in Table 1. (H-2^b is the haplotype of the primed spleen cells used in the fusion; H-2^k is that of the thymoma cells to which they were fused.) Precipitation with anti-mIg reagents, including anti-IgM (MOPC 104E) and anti-IgD (Sera Labs), or with sheep anti-mouse whole serum, also failed to remove the SRBC-binding molecule from the tritiated A1 supernatant.

SRBC which had been allowed to bind the tritiated A1 factor were washed, dissolved in SDS in reducing conditions and electrophoresed on 10% and 15% polyacrylamide gels⁴ (Fig.

Table 2 Binding to red cells of supernatant of T-hybrid line A1 after incorporation of ³H-leucine

Supernatant	Treatment	c.p.m. bound by red cells of:				
		Sheep	Mouse	Ox	Rabbit	Donkey
BW 5147	—	82	71	64	63	87
A1	—	640	80	83	240	106
A1	Anti-H-2 ^b					
	precipitation	166				
A1	Anti-H-2 ^k					
	precipitation	519				

50 μ l of supernatants of the A1 hybrid line or of the thymoma BW 5147, after labelling with ³H-leucine as described in the text, were reacted with 10 μ l 10% red cells of the species shown for 1 h at room temperature. The red cells were then washed 3 times and counted in a β -counter. Where indicated, A1 supernatant was precipitated, before the binding assay, by addition of 10 μ l undiluted anti-H-2 antiserum to 50 μ l supernatant, followed after 1 h by 150 μ l sheep anti-mouse whole serum. Anti-H-2^b antiserum was C3H anti-C3H.B10; anti-H-2^k was C57BL/10 anti-C3H. Results are means of duplicate tests.

1a, b). This revealed two polypeptide chains, a large chain which showed an apparent MW of about 85,000 and a smaller chain of about 25,000. Similar gel profiles were obtained in the absence of 2-mercaptoethanol (ME), indicating that the chains are not linked by disulphide bonds. If the A1 supernatant was first reacted with anti-H-2^b antiserum, followed by precipitation with sheep anti-mouse whole serum, both chains were removed; reaction with anti-H-2^k antiserum and sheep anti-mouse whole serum failed to precipitate either chain (Fig. 1c). From these observations, it seems that the SRBC-binding molecule produced by the A1 hybrid line is composed of two types of polypeptide chain, one or both of which carries determinants coded by genes in the H-2 complex of the primed spleen cells used in the fusion.

Attempts were then made to identify the SRBC-binding molecule in lysates of the labelled A1 cells. After incorporation of ³H-leucine as above, A1 cells were dissolved in the non-ionic detergent Nonidet P40 (NP40). Nuclei were spun down and the solubilised material tested first for binding to glutaraldehyde-fixed red cells (the fixation was such as to prevent lysis in NP40, but leave red cell antigens available, as evidenced by binding of anti-red cell antibodies). Table 3 shows that the NP40-extracted material from A1 bound to SRBC specifically; however, binding was not appreciably reduced by precipitation of the extracts with anti-H-2 antisera. Furthermore, when analysed by SDS-PAGE, the NP40 lysate of A1, absorbed onto fixed SRBC, showed a single peak of radioactivity, corresponding to the large chain of the A1 supernatant molecule (Fig. 1d). This peak persisted after precipitation of the lysate with anti-H-2^b antiserum and sheep anti-mouse whole serum. These findings imply that the H-2 determinants of the supernatant factor secreted by A1 are associated only with its smaller polypeptide chain. The absence of the latter from NP40 extracts suggests that the large and small chains may be assembled only when the molecule is secreted and that within the cytoplasm they may exist as separate pools.

Table 3 Binding to red cells of NP40 extracts of T-hybrid line A1 after incorporation of ³H-leucine

Extract	Treatment	c.p.m. bound by red cells of:		
		Sheep	Ox	Donkey
BW 5147	—	125	85	135
A1	—	520	116	143
A1	Anti-H-2 ^b			
	precipitation	405	91	76
A1	Anti-H-2 ^k			
	precipitation	476	82	89

Hybrid line A1 and thymoma BW 5147 were internally labelled by growth in ³H-leucine as described in the text. Extracts were prepared by dissolving 2 × 10⁷ labelled cells in 500 μ l 1% NP40 and aliquots of 25 μ l were tested for binding to 50 μ l 2% red cells previously fixed in 0.25% glutaraldehyde. Precipitation with anti-H-2 antisera was as in Table 2. Results are means of duplicates.

Although the structure of the A1 suppressor factor is reminiscent of 7S immunoglobulin, the chains are not disulphide linked and moreover lack the antigenic characteristics of either Ig heavy or light chains. The only antigenic determinants so far detected on the suppressor are those of the H-2 complex. The observations with labelled molecules extracted from A1 cells with NP40 indicate that the large chain is able to bind specifically to SRBC, but lacks both Ig and H-2 determinants. We conclude, therefore, that the H-2 specificities detectable on the secreted factor are probably associated with the 25,000-MW chain. As yet there is no evidence that the latter has a role in binding to SRBC.

The similarity in general properties between the A1 factor and antigen-specific T-cell factors has already been noted, in particular, the features of specific antigen binding together with H-2 association. A notable difference is the molecular weight, as

the MWs of the factors obtained by sonication of suppressor T cells³ or from T-cell supernatants² have been found to lie in the range of 35–55,000. It may be that the MWs of factors reported hitherto have been underestimated, perhaps due to partial proteolytic digestion before sizing.

The indication that H-2 specificities are carried by the smaller chain of the A1 factor is of particular interest. We have not yet mapped the sub-region of H-2 coding for this chain, but antigen-specific suppressor factors obtained from T cells³ or from another T-hybrid line⁵ carry Ia determinants of the I-J sub-region. The size of the small chain of the A1 factor, about 25,000, would be consistent with an origin in the I region, as mouse Ia molecules have chains of MWs about 25,000 and 35,000 (ref. 6). Although the large chain of the factor carries a binding site for SRBC, it may be the role of the H-2-derived chain to provide the suppressive activity of the factor. The importance of the binding site would then be to ensure a high local concentration of factor at the surface of antigen-binding lymphocytes and hence specificity of suppression¹.

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Crossreacting determinants in the C-terminal region of trypanosome variant surface antigens

ANTIGENIC variation seems to be the primary mechanism enabling the pathogenic African trypanosomes to evade the immune systems of their mammalian hosts (reviewed in refs 1–3). Antigenic variation is mediated through sequential expression of an extensive repertoire of variant surface glycoproteins (VSGs). Several VSGs have been purified from cloned variants of *Trypanosoma brucei*^{4,5}. The diversity in amino acid composition, conformational features⁶ and N-terminal amino acid sequences⁷ implies extensive structural diversity in VSGs so far studied. Earlier studies failed to identify immunological crossreactivity between VSGs when purified or whilst on the surface of living trypanosomes. Some time ago, Dr Frans van Knapen and I, while performing enzyme-linked immunoabsorbant assays, were puzzled to observe extensive crossreactions between two purified VSGs and a wide range of antisera to different trypanosome species (unpublished experiments). A recent study by Barbet and McGuire⁸ provided additional evidence for the presence of strongly crossreacting determinants on a wide range of VSGs. In several cases, the VSGs examined by Barbet and McGuire originated from clones which I previously prepared and examined. I have therefore re-examined several VSGs and their corresponding antisera by radioimmunoassay. The results reported here are consistent with those of Barbet and McGuire. Additional observations suggest that the crossreacting determinants are located towards the C-terminal end of the VSG.

As no rational basis for classification of VSGs exists, they are referred to by the stock numbers of the trypanosome clone from which they were obtained, as previously described⁴. Figure 1 shows the titration, by radioimmunoassay, of antisera of seven specificities against four variant antigens purified from randomly selected clones of *T. brucei* strain 427 (ref. 4). It is clear that VSGs 55, 221 and 121 may be substantially precipitated by certain heterologous antisera; VSG 55 (Fig. 1a) is most

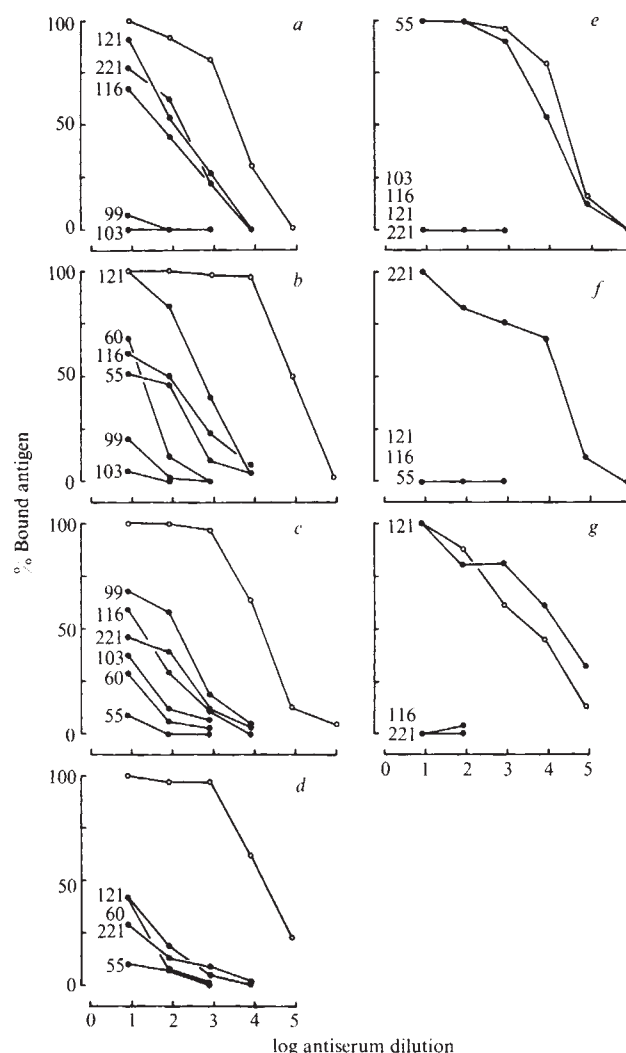


Fig. 1 Immunoprecipitation of four VSGs and three N-terminal tryptic fragments by homologous and heterologous antisera. The antigens are VSGs 55 (a); 221 (b); 121 (c); 116 (d); and N-terminal fragments from VSGs 55 (e); 221 (f); and 121 (g). ○, Homologous antisera; ●, heterologous antisera. Antigens were labelled by the chloramine-T method to a specific activity between 1,000 and 10,000 c.p.m. per ng. Antisera were raised in 2–4-kg New Zealand White rabbits by primary subcutaneous injection at four sites of a total of 1 mg purified VSG in an emulsion of 0.2 ml saline per 0.2 ml Freund's complete adjuvant. Two further subcutaneous injections of 0.5–1 mg VSG in saline were given at 10-d intervals. Sera were obtained 7–10 d after the third or subsequent injections of antigen and were lyophilised and stored under nitrogen at -20°C . Radioimmunoassay was performed by incubating (2 h at 37°C in a total volume of 0.065 ml phosphate-buffered saline¹² containing 4 mg ml⁻¹ bovine albumin) 1 ng antigen with antisera dilutions prepared in appropriate dilutions of normal rabbit serum to maintain a constant amount of rabbit serum in each assay. Antigen-antibody complexes were precipitated with goat anti-rabbit IgG (Miles) and counted. Antiserum dilution is plotted against the percentage of bound antigen, after correction for nonspecific binding, taking the maximal precipitation by homologous antiserum as the 100% value. Maximal precipitation was generally equivalent to 95–100% of acid-precipitable radioactivity.

effectively precipitated by heterologous antisera 116, 121 and 221. However, an eightfold dilution was required to approach 100% precipitation. At this concentration neither antisera 99 nor 103 caused significant precipitation; 121 was the only heterologous antiserum which totally precipitated VSG 221 (Fig. 1b). The concentrations of heterologous antisera required to precipitate 50% of VSG 221 or 121 were 100-fold to 1,000-fold higher than the effective concentration of homologous antiserum. VSG 116 (Fig. 1d) was even less effectively precipitated by the four heterologous antisera tested.

The extent of heterologous precipitation demonstrated in these experiments is more variable and considerably less than that experienced by Barbet and McGuire⁸. Several factors may combine to explain both this discrepancy and my own earlier failure, and possibly that of others, to detect significant cross-reactions. In agreement with our previous experiences, Barbet and McGuire were unable to demonstrate crossreactions by gel immunodiffusion or by reaction of living trypanosomes with fluorescent antisera. These were the only techniques which I routinely used for screening the antigenic specificities of trypanosomes and purified VSGs. Only minimal use had been made of radioimmunoassay procedures and these had been performed using ³⁵S-formylmethionine sulphone-labelled⁹ antigens having only 1% of the specific activity of the iodinated antigens used in the current experiments. Considering the low specific activity and small range of VSGs and antisera previously used, it is not surprising that we did not detect crossreactions. Recent experience suggests that the immunisation protocol used by Barbet and McGuire, particularly the use of very low amounts of VSG, is an additional factor in obtaining strongly crossreacting antisera.

Our findings^{6,10} that native VSGs may be selectively cleaved by trypsin and the resultant large and small N-terminal and C-terminal fragments separated in the absence of denaturing agents, offered the possibility of determining whether the crossreacting determinants were localised in one region of the molecule. Large undenatured N-terminal tryptic fragments of molecular weights 52,000, 41,000 and 44,000 were purified from VSGs 55, 121 and 221 respectively. Antisera were raised against the fragments from VSGs 55 and 121. The three fragments were iodinated and their precipitation by heterologous and homologous antisera was investigated.

The results given in Fig. 1 e, f, g show that the tryptic fragments from VSGs 55 and 121 were efficiently precipitated by homologous antisera from rabbits immunised with either the corresponding fragment or the intact VSG. The fragment from VSG 221 was efficiently precipitated by antiserum against intact VSG 221. There was no precipitation of any N-terminal fragment by heterologous antisera, in striking contrast to the results obtained with the intact VSGs. A subsequent experiment with more strongly crossreacting antisera confirmed these results. Additionally, antisera raised against the N-terminal fragments of VSGs 55 and 121 were unable to precipitate intact VSG 221. All these results strongly suggest that the crossreacting determinants are not located in N-terminal fragments representing up to 80% of the intact molecule. However, crossreacting determinants might be formed by amino acid sequences in both N-terminal and C-terminal domains being brought into proximity by the overall conformation of the intact glycoprotein. In view of the ease with which the two domains dissociate on tryptic cleavage, this seems unlikely.

Confirmation that the C-terminal fragment was the unique location of crossreactivity, was sought by inhibition studies using a glycopeptide fraction representing the C-terminal 17,000 molecular weight initial cleavage product of VSG 121 (equivalent to VSG 52 in ref. 6). Carbohydrate is found only in the C-terminal 25% of VSG 121 (refs 6, 10). Following trypsin digestion of VSG 121, a glycopeptide fraction was purified by absorption on concanavalin A-Sepharose, followed by elution and rechromatography on Sephacryl-S200. A single peak of 14,000 apparent molecular weight was recovered from the Sephacryl column. At a 10-fold molar excess, this undenatured

C-terminal glycopeptide fraction was able to inhibit by 80% the immunoprecipitation of intact VSG 55 by antiserum 121 (Table 1). A 540-fold molar excess of the N-terminal 52,000 molecular weight fragment of VSG 55 did not inhibit cross precipitation: neither did a 160-fold molar excess of the N-terminal 41,000 molecular weight fragment of VSG 121. These results suggest that the crossreactions can be attributed to the C-terminal regions of VSGs 55 and 121.

The second section of Table 1 shows the competitive effects of various amounts of unlabelled intact VSGs on the precipitation of radioactive VSG 55 by a dilution of antiserum 121 which gave 60% precipitation of VSG 55 in uninhibited controls. The effectiveness of different unlabelled competitor VSGs as inhibitors varied; VSGs 55, 121 and 60 were most effective, VSGs 221 and 116 were less effective and VSG 118 at a 1,000-fold excess was only partly competitive. The reason for these variations is not apparent. One implication is that the crossreacting determinants are not shared by all VSGs. In a region the size of the approximately 150 amino acid C-terminal domain, there might be several antigenic determinants which are shared to different extents by different groups of VSGs. On the other hand, the inhibition experiments of Barbet and McGuire⁸ suggest a much greater uniformity of crossreactivity between all the VSGs tested by them. This discrepancy may reflect the relatively few VSGs now being studied in either laboratory. It is possible that immunological crossreactivity will identify the existence of different VSG subgroups. The use of monoclonal antibodies¹¹ might clarify the situation.

The C-terminal domain of VSG 121 contains all the carbohydrate; could carbohydrate be responsible for the immunological crossreactivity? This is not easy to confirm, but three relevant observations have been made. First, concanavalin A did not block the crossreactivity between VSG 55 and antiserum 121 (Table 1). Second, VSG 118, which was the least effective inhibitor of crossreactivity, contains more carbohydrate (17%

Table 1 Inhibition of immunoprecipitation of VSG 55 by antiserum to VSG 121

Competitor (unlabelled)	ng per assay	% Inhibition of precipitation of radioactive antigen
55 f52	430	5
	43	2
121 f41	100	5
	10	0
121 f17	260	100
	26	100
	2.6	80
VSG 55	100	98
	10	74
	1	8
VSG 121	260	100
	26	100
	2.6	22
VSG 221	100	95
	10	22
	1	5
VSG 60	10	100
	1	31
VSG 116	100	95
	10	35
	1	1
VSG 118	1000	27
	100	10
	10	0
Concanavalin A	100	0
	10	0

Unlabelled competitors were diluted in PBS containing 2 mg ml⁻¹ bovine albumin. Assay conditions were otherwise as described for Fig. 1 except that the total incubation volume was 0.085 ml. 55 f52 and 121 f41 are the N-terminal tryptic fragments from VSGs 55 and 121. 121 f17 is a C-terminal fragment from VSG 121.

by weight) than any other VSG studied. Third, one experiment was performed in which the 121 C-terminal precipitation-blocking glycopeptide was treated with *Streptomyces griseus* endoglycosidase H (Miles). After treatment, the C-terminal fragment was repurified by passage through a column of concanavalin A-Sepharose, on which it was no longer retained. After removal of the oligosaccharide side chain, blocking activity was not eliminated but was reduced 10-fold. This reduction could be due to conformational changes arising during the several repurification steps which were used after glycosidase treatment. The possible role of carbohydrate in cross-reactivity needs further investigation.

From the evidence of its susceptibility to proteolytic cleavage⁶, the VSG C-terminal polypeptide region may be rather loosely folded. We must therefore also consider that slight denaturation may expose antigenic determinants in this area which are normally masked by the native configuration of the polypeptide.

It might reasonably be assumed that VSGs have evolved by gene duplication and mutation or are made by some kind of gene-splicing mechanism. Therefore, crossreactivity is perhaps not surprising. Another argument for some conservation of structure is that all VSGs must presumably attach to the plasma membrane in a similar manner. The crossreacting determinants are apparently masked on living trypanosomes⁸ and so is the C-terminal region of the VSG¹⁰. These facts do not greatly encourage us to believe that crossreacting determinants could provide a basis for immunisation, although this possibility will undoubtedly be explored. The significance of crossreactivity must be clarified by immunochemical and amino acid sequence studies of a range of VSGs.

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Dexamethasone-induced adhesion in hepatoma cells: the role of plasminogen activator

TRANSFORMED CELLS are frequently less adhesive than normal cells. This decrease in adhesion has been associated with the loss of specific cell surface proteins, changes in morphology, alterations in the underlying cytoskeleton and the increased activity of the serine protease, plasminogen activator¹⁻³. Hepatoma tissue culture (HTC) cells exhibit increased levels of adhesion to glass^{4,5} and decreased plasminogen activator activity^{6,7} when incubated in the presence of the synthetic glucocorticoid, dexamethasone. Plasminogen activator converts plasminogen in serum to plasmin. It has been shown that plasmin causes the reversible dissociation of intracellular actin-containing cables in anchorage-dependent rat embryo cells⁸ and may also be partially responsible for decreased adhesion of Rous sarcoma virus-transformed chicken embryo fibroblasts³. Cell-

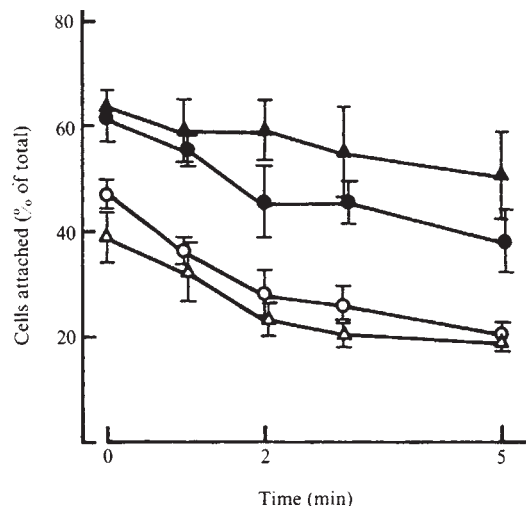


Fig. 1 Wild-type HTC cells were incubated overnight in radioactive medium containing 3% plasminogen-depleted fetal calf serum in the presence (●) or absence (○) of 0.1 μ M dexamethasone, or in the same medium reconstituted with 4.8 μ g ml⁻¹ plasminogen in the presence (▲) or absence (△) of 0.1 μ M dexamethasone. The assay conditions were the same as for cells incubated in serum-containing medium (see legend to Table 1). Plasminogen was obtained from outdated human plasma by affinity chromatography using lysine-Sepharose 4B (Pharmacia) followed by elution with ϵ -aminocaproic acid as described by Deutsch and Mertz¹⁶. Plasminogen-depleted serum was prepared by passing fetal calf serum through a lysine-Sepharose column and collecting the non-adsorbed fraction. This was acid treated to reduce the activity of protease inhibitors¹⁷ and filter sterilised (0.45 μ m pore size).

substrate adhesion presumably requires specific cell surface glycoproteins and intact cytoskeletal elements^{9,10}. It is possible that plasmin acts either to modify 'adhesive' proteins on the cell surface or to disrupt linkages between these surface components and the underlying cytoskeleton. We have used variant HTC cells selectively resistant to the dexamethasone inhibition of plasminogen activator⁶ to test the hypothesis that hormonal induction of cell adhesion is secondary to the inhibition of plasminogen activator which in turn allows the accumulation of the glycoproteins necessary for adhesion. We have modified cell adhesion assay techniques^{5,11} to provide a sensitive measure of cell-substrate adhesion for both wild-type and variant HTC cells in the presence and absence of serum and plasminogen. We report here that plasminogen activator does not have an important role in the hormonal regulation of HTC cell adhesiveness.

HTC cells were routinely grown in spinner culture without antibiotics in Eagle's minimal essential medium, modified to contain 2 mM glutamine, 5% each heat-inactivated fetal calf and calf serum (Grand Island Biological), 5.8 mM NaHCO₃ and 50 mM Tricine (Sigma). Variant HTC cell lines fully resistant to the dexamethasone inhibition of plasminogen activator were isolated using an agar-fibrin overlay technique to detect plasminogen-activator production by individual colonies of cells⁶. Glucocorticoid-receptor function was determined to be normal in variant cell lines on the basis of normal tyrosine aminotransferase induction by dexamethasone, assayed as described by Spencer and Gelehrter¹².

Wild-type HTC cells incubated for 18 h in serum-containing medium in the presence of 0.1 μ M dexamethasone typically show a twofold increase in the per cent of cells remaining attached to a glass surface after 5 min of shaking, when compared to untreated cells (Table 1). After cells have been allowed to settle and attach, differences in adhesion between dexamethasone-treated and untreated cells are evident. In the presence of dexamethasone there are fewer loosely adherent cells initially, and those which remain after settling are more adherent than those in the untreated cell population. In the conditions of the assay, maximal detachment occurs within 2-5 min. We find that dexamethasone also induces adhesion in variant HTC cells which are resistant to the dexamethasone

inhibition of plasminogen activator (Table 1). Although variant C cells are less adhesive than the wild-type HTC cells, there is a twofold increase in the adhesion of dexamethasone-treated cells incubated in the presence of serum. Two other HTC cell variants, A and B, which are also fully resistant to the dexamethasone inhibition of plasminogen activator, have similar dexamethasone-induced adhesion (data not shown). These results suggest that the mechanism by which dexamethasone increases cell-substrate adhesion does not depend on the inhibition of plasminogen activator.

When incubated in serum-free medium, wild-type HTC cells again show a twofold increase in adhesion in the presence of dexamethasone (Table 1). Variant C cells are less adhesive than the wild-type HTC cells in these conditions as well, but also show a twofold increase in adhesion when incubated in the presence of dexamethasone (Table 1). Likewise, variants A and B consistently exhibit dexamethasone-induced adhesion in the absence of serum. In both wild-type and variant HTC cells, the induction of adhesion by dexamethasone occurs to a similar extent in the presence and absence of serum.

We have also directly tested the effects of plasminogen, the substrate for plasminogen activator, on the dexamethasone induction of adhesion in wild-type and variant HTC cells in serum-free conditions. If the formation of plasmin from plasminogen prevents the accumulation of cell surface proteins required for adhesion, then the addition of plasminogen to serum-free medium should decrease or prevent dexamethasone induction of adhesion in the variant cell lines. Instead, we find that dexamethasone induces increased levels of adhesion in both wild-type and variant cells which have been incubated for 18 h in

medium containing plasminogen (data not shown). This also suggests that plasminogen-activator activity does not modulate the adhesive response to dexamethasone.

In conditions in which plasminogen is the only serum factor missing, wild-type cells exhibit a twofold increase in adhesion in the presence of dexamethasone (Fig. 1). When cells are incubated for 18 h in medium containing depleted serum which has been reconstituted with plasminogen, a similar, although somewhat greater, increase in adhesion is measured in dexamethasone-treated cells. In the absence of dexamethasone, cell adhesion is the same whether the medium contains plasminogen-depleted or reconstituted serum. These results also suggest that plasminogen-activator production does not have a major role in cell-substrate adhesion.

Note that the optimal assay conditions differ in the presence and absence of serum. When serum is present, cell-substrate adhesion may depend on interactions between the cell surface and serum proteins which are adsorbed to the glass¹³. This might be relevant to our observation that HTC cells incubated in serum-containing medium require a longer time to form stable adhesive bonds to a substrate than do cells in serum-free medium containing bovine serum albumin (BSA). It has been proposed that cell surface proteases on BHK cells modify substrate-adsorbed proteins during the process of cell adhesion¹⁴. Although other cell surface proteases may be involved in cell-substrate adhesion, the nearly identical adhesiveness of untreated HTC cells in fetal calf serum, plasminogen-depleted serum and reconstituted serum provides additional evidence which tends to rule out any important role for plasminogen activator or plasmin in this process.

Recently, a class of dexamethasone-induced proteins, tentatively described as sialoglycoprotein on the basis of electrophoretic properties in a two-dimensional gel system, has been localised on the surface of HTC cells¹⁵. It is possible that such proteins could confer increased adhesive properties on hormone-treated cells. Our data suggest that dexamethasone regulation of cell adhesion in HTC cells probably operates primarily through the synthesis of surface glycoproteins and that this process is not, in turn, regulated by the inhibition of plasminogen-activator activity.

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Wild-type or variant HTC cells were collected from spinner culture during the logarithmic stage of growth. Cells were resuspended in medium containing 3% fetal calf serum, $0.1 \mu\text{Ci ml}^{-1}$, ^3H -amino acid mixture (NEN) and $50 \mu\text{g ml}^{-1}$ neomycin, in the presence or absence of $0.1 \mu\text{M}$ dexamethasone (Merck). After an 18-h incubation at 37°C in a gyrotory shaker-waterbath at 80 r.p.m., the cells were centrifuged, and the cell pellet washed twice in nonradioactive medium and resuspended to a final density of 10^6 cells ml^{-1} in nonradioactive medium. Cell viability, as measured by trypan blue exclusion, was always greater than 90%. One-ml aliquots of the cell suspension were distributed into untreated borosilicate glass scintillation vials and incubated without shaking for 2 h at 37°C . At the end of the incubation period, the cells completely covered the surface of the vial with little or no piling up of cells. The medium was removed and 1 ml calcium- and magnesium-free phosphate-buffered saline (PBS), pH 7.3, added carefully down the side of each vial. The vials were then subjected to a shearing force of 100 strokes min^{-1} in a Dubnoff reciprocating shaker-waterbath (5-cm stroke) at 37°C . At 0, 2 and 5-min exposure to this reciprocating force, duplicate vials were removed and the PBS aspirated. Cells adhering to the glass scintillation vials were lysed with $0.1 \text{ ml } 1 \text{ M KOH}$ and the lysate neutralised with $0.1 \text{ ml } 1 \text{ M HCl}$. Radioactivity was assayed in a scintillation spectrometer after addition of 5 ml ACS scintillation fluid (Amersham-Searle). In control experiments not shown, cells were removed from the surface of vials by trypsin following the 5-min shaking period. These cells were viable by the criterion of trypan blue exclusion and the cell counts correlated with direct measurements of radioactivity in each vial. Radioactivity of the initial cell inoculum was determined from 1-ml aliquots of the cell suspension which were centrifuged, the cell pellets lysed in $0.1 \text{ ml } 1 \text{ M KOH}$ and the lysate neutralised in $0.1 \text{ ml } 1 \text{ M HCl}$. The per cent of initial labelled cell inoculum remaining after shaking (average of duplicate vials) has been used as a measure of cell adhesiveness. The data presented represent the mean $\pm$ s.e.m. calculated from several experiments. In experiments carried out in serum-free medium (supplemented with 0.1% BSA), the assay was modified to include a 1-h incubation period for cell attachment and a reciprocating force of 140 strokes min^{-1} .

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Glucocorticoids regulate the concentration of 1,25-dihydroxycholecalciferol receptors in bone

THE results of a study of high-affinity glucocorticoid and 1,25-dihydroxycholecalciferol ($1,25(\text{OH})_2\text{D}_3$) binding in cytosol from fetal rat calvaria cultured for up to 48 h at 37°C are presented. The binding of glucocorticoids was found to be stable in short-term culture, whereas that for $1,25(\text{OH})_2\text{D}_3$ declined rapidly, was almost undetectable at 24 h and had disappeared by 48 h. Furthermore, low concentrations of cortisol (10^{-8} to 10^{-6} M) added to the culture medium had a dramatic effect on the persistence of high affinity $1,25(\text{OH})_2\text{D}_3$ binding. In the presence of cortisol there was dose-dependent preservation of most of the binding of $1,25(\text{OH})_2\text{D}_3$ in the cytosol. $1,25(\text{OH})_2\text{D}_3$ is a potent agent in inducing bone resorption *in vitro* and these data suggest that glucocorticoids may exert part of their effects on calcium metabolism and bone *in vivo* by directly regulating the turnover of cytosol $1,25(\text{OH})_2\text{D}_3$ receptors in one or more types of bone cell.

We recently developed a simple method for the detection of high-affinity glucocorticoid binding ($K_D \approx 5 \times 10^{-9}$ mol l⁻¹) in fetal rat calvaria cytosol¹ and, by applying a similar method we also detected binding of similar affinity ($K_D \approx 2.3 \times 10^{-9}$ mol l⁻¹) for $1,25(\text{OH})_2\text{D}_3$ (ref. 2). Glucocorticoid receptors had been previously demonstrated in bone cells by Feldman *et al.*³, and $1,25(\text{OH})_2\text{D}_3$ receptors in bone calvaria cytosol in a preliminary report by Kream *et al.*⁴. In the latter study bone cytosol receptors for $1,25(\text{OH})_2\text{D}_3$ were shown by sucrose density gradient centrifugation to have sedimentation characteristics (3.5S) similar to the better-defined $1,25(\text{OH})_2\text{D}_3$ receptors of intestine. We have confirmed this finding (data not shown). In our method, in contrast to others, the equilibrium was set up first in the intact calvaria at 4°C and then the cytosol prepared by sonication and ultracentrifugation, and bound separated from free steroid with dextran-charcoal. The advantages of this method are its simplicity, elimination of the risk of degradation of receptors and loss of cells during collagenase digestion, and the establishment of binding to intracellular receptors at their physiological concentrations.

The bound:free ratio in the cytosol using tracer-labelled glucocorticoid or $1,25(\text{OH})_2\text{D}_3$ (10^{-9} mol l⁻¹) is as high as 60% displacing down to 5–10% with 100-fold excess of unlabelled steroid. Cross-competition experiments with up to 1,000-fold excess competing steroid showed that the glucocorticoid and $1,25(\text{OH})_2\text{D}_3$ receptors are quite distinct from one another.

Although only a rough assessment of K_D or binding-site concentration is possible by this method, it is highly reproducible both for glucocorticoids and $1,25(\text{OH})_2\text{D}_3$, and we have found the binding characteristics to be similar to those obtained in cytosol prepared by the methods of Feldman *et al.*³ for glucocorticoids and Kream *et al.*⁴ for $1,25(\text{OH})_2\text{D}_3$ respectively. The reproducibility is due in part to the very slow rate of dissociation of glucocorticoids and $1,25(\text{OH})_2\text{D}_3$ from their respective receptors as a consequence of dilution after cell disruption, which seems not to be significant over the 1–4 h elapsing between sonication of calvaria and the final charcoal separation step. This simple method is suitable for semi-quantitative study of changes in overall receptor content during culture of intact bone.

A marked difference was observed between the stability in short-term organ culture of the rat calvaria cytosol receptors for glucocorticoids, and $1,25(\text{OH})_2\text{D}_3$ (Fig. 1). High-affinity glucocorticoid binding persisted for 24 h unchanged, while that for $1,25(\text{OH})_2\text{D}_3$ diminished progressively after 2 h in culture and was minimal by 24 h. This marked difference suggested to us that $1,25(\text{OH})_2\text{D}_3$ cytosol receptor content is being actively maintained by some agent or agents *in vivo* that are missing from the culture medium.

Oestradiol or testosterone (each at 10^{-6} M) had no effect on the rate of disappearance of $1,25(\text{OH})_2\text{D}_3$ binding. These steroids lack specific cytosol receptors^{3,5} in rat calvaria. However, a marked and dose-dependent effect of the glucocorticoid cortisol was demonstrated over the range 10^{-8} to 10^{-6} M (Fig. 2). There was no consistent effect at 2 h, but a dramatic preservation of $1,25(\text{OH})_2\text{D}_3$ binding at 6, 24 and 48 h. After culture for 48 h in the absence of cortisol there was no residual high-affinity $1,25(\text{OH})_2\text{D}_3$ binding, while in the presence of cortisol (10^{-6} M) binding was preserved at the level seen after 24 h of culture with cortisol. When calvaria were cultured for the first 24 h without steroid, and cortisol (10^{-6} M) subsequently added and culture continued for a second 24 h, binding of $1,25(\text{OH})_2\text{D}_3$ was not restored but showed no further fall over the second 24 h.

When other glucocorticoids were tested in this system at 10^{-7} M for 24 h, they all significantly preserved binding of $1,25(\text{OH})_2\text{D}_3$. Their relative efficacies in preserving $1,25(\text{OH})_2\text{D}_3$ were closely related to their relative potencies as glucocorticoids and affinities for the glucocorticoid receptors (triamcinolone acetonide > dexamethasone > cortisol > corticosterone > progesterone).

These findings strongly suggest that glucocorticoids are important for maintaining the content of cytosol receptors for $1,25(\text{OH})_2\text{D}_3$ in bone, and favour an inhibition of degradation of existing $1,25(\text{OH})_2\text{D}_3$ receptors, rather than the induction of synthesis of new receptors. Our present data do not define the mechanism, but suggest that it is mediated by binding of the glucocorticoid to its receptor. It is of potential physiological importance since it occurs at physiological concentrations of glucocorticoids.

The effects of glucocorticoids on bone are of clinical significance, being involved in the rapid and severe bone loss seen in Cushing's syndrome and in patients on steroid therapy. In the presence of excess glucocorticoids, intestinal calcium

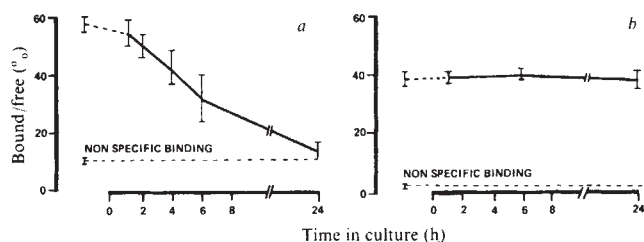


Fig. 1 Comparison of persistence of fetal rat calvaria glucocorticoid (b) and $1,25(\text{OH})_2\text{D}_3$ receptors (a) in short-term organ culture at 37°C. Cells were cultured on stainless steel grids, two half calvaria per grid, in sterile plastic Petri dishes with 0.3 ml culture medium under and in contact with each grid, in an atmosphere of 5% CO_2 in air at 37°C. Culture medium was medium 199 (Flow Labs) with 1.2% NaHCO_3 , 40 $\mu\text{g ml}^{-1}$ gentamicin, 1 mM glutamine, 0.15 mg ml^{-1} ascorbic acid and 5% heat-inactivated rabbit serum (fluorometry revealed up to 1.45×10^{-8} M corticosterone in medium). All calvaria were kept at 4°C except during the period of culture (0, 1, 2, 4, 6 or 24 h). After culture they were washed and again left in fresh culture medium at 4°C. On completing this for the last set of calvaria, they were washed this time in incubation medium and incubated overnight in lots of four half-calvaria at 4°C in 0.5 ml of this solution containing 1×10^{-9} M ^3H -labelled, with or without 100-fold excess unlabelled steroid. For detecting the glucocorticoid receptor 1,2,4(N)- ^3H -triamcinolone acetonide (Radiochemical Centre, Amersham, TRK 485, specific activity 29 Ci mmol⁻¹) was used as ligand because of its high affinity, and for the $1,25(\text{OH})_2\text{D}_3$ receptor 23,24(N)- ^3H - $1,25(\text{OH})_2\text{D}_3$ (94 Ci mmol Amersham TRK 588) was used in place of the lower specific activity compound used in our earlier studies². After overnight incubation, calvaria were washed again, sonicated, and the sonicate ultracentrifuged (100,000g for 30 min) bound and free separated with charcoal, and bound and total radioactivity counted^{1,2}. Mean and range of observations of bound:free ratio from three separate experiments are indicated (six observations, each the mean of duplicates per time-point, for both steroids). The first data points are for controls at 4°C.

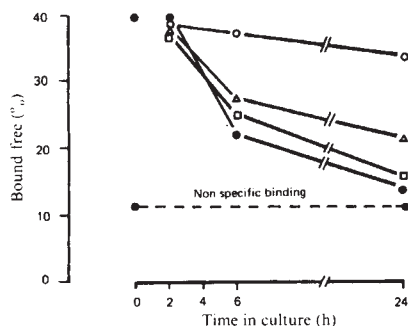


Fig. 2 Representative experiment showing the effect of including cortisol in the incubation medium on the persistence of $1,25(\text{OH})_2\text{D}_3$ receptors in fetal rat calvaria. Each point is the mean of duplicate observations in cytosol from four half-calvaria. Incubations with 100-fold excess $1,25(\text{OH})_2\text{D}_3$ indicate nonspecific binding which remained unchanged. In the absence of added cortisol (●) there was progressive loss of high affinity binding to very low levels at 24 h. ○, ▼ and □ indicate results in the presence of 10^{-6} M, 10^{-7} M and 10^{-8} M cortisol respectively.

absorption⁶ and bone formation are reduced and bone resorption increased⁷. Numerous studies both *in vivo* and *in vitro*, in animals and man however have not fully elucidated the mechanism of these effects⁸⁻¹², although recent studies indicate that one action of glucocorticoids in birds¹³ and possibly in mammals¹⁴ is to stimulate renal 1α -hydroxylase activity. Our data indicate for the first time that glucocorticoids may influence the stability of cytosol $1,25(\text{OH})_2\text{D}_3$ receptors in bone. From these experiments we cannot tell if this effect is specific to one population of bone cells or a general one affecting both osteoblasts and osteoclasts. There is evidence that both types of cell respond to $1,25(\text{OH})_2\text{D}_3$ at concentrations of 10^{-9} M (ref. 15). An augmentation of $1,25(\text{OH})_2\text{D}_3$ receptors in osteoclasts which would result from retarding their degradation might enhance bone resorption, since this metabolite of vitamin D is among the most potent bone-resorbing agents known^{16,17}.

Our studies indicate the need for a thorough examination of the effects of glucocorticoids on $1,25(\text{OH})_2\text{D}_3$ and other receptors in cytosol and nuclei in isolated bone cell populations and in intestinal cells.

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Is the benzodiazepine receptor coupled to a chloride anion channel?

HIGH affinity saturable binding sites for benzodiazepines (BZs) in mammalian brain have been described¹. These sites show stereospecificity, organ and subcellular distribution, and structure-activity relationships reminiscent of other synaptic membrane receptors which have been characterised by ligand binding^{2,3}. The BZ receptor is so highly selective that no known putative neurotransmitters interact with it, thus raising the possibility that the brain contains another unidentified endogenous ligand^{4,5}. Recently, Tallman *et al.* have reported that γ -aminobutyric acid (GABA) specifically enhances the binding of ^3H -diazepam to rat brain membranes⁶. Considerable biochemical and neurophysiological evidence suggests that GABA may be intimately involved in the mechanism of action of BZs^{7-10,18}. In several neurotransmitter systems, ions seem to have specific effects on the binding of synaptic receptor ligands, presumably because of the intimate association of the receptor with an ion channel. For example, binding of opiate agonists and antagonists is differentially affected by sodium ion¹¹, and Na^+ conductance is altered by opiate agonists¹². Similarly, specific effects of anions on the receptors for strychnine¹³ and GABA¹⁴ are correlated with observations that the neurophysiological action of these receptors is dependent on alterations in chloride ion conductance^{15,16}. We now report the specific effect of chloride and other anions in enhancing BZ binding to its receptor. We infer that the BZ receptor (recognition site) may be

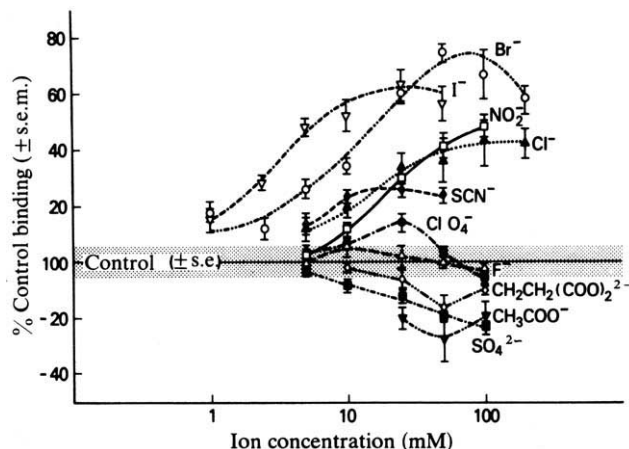


Fig. 1 Effect of various anions on ^3H -diazepam binding. Male rats (200-250 g) were decapitated. Cerebral cortices were washed quickly in ice-cold Tris-maleate buffer (50 mM, pH 7.6 at 0°C), homogenised in 100 vol of the same buffer (w/v) using a Brinkmann Polytron for 20 s and centrifuged at 20,000 g for 20 min at 7°C (J-21 Beckman centrifuge). Pellets were resuspended by Polytron in 100 vol of buffer. Radioreceptor assay incubations contained 0.5 ml of membranes, 0.3 nM ^3H -diazepam ($\sim 80 \text{ Ci mmol}^{-1}$, NEN), 0.1 ml of the added salt solution (or distilled water as control) and buffer in a total volume of 1 ml. After incubation for 30-45 min at 0°C , tubes were rapidly filtered through glass fibre filters (GF/B, Whatman) *in vacuo*. Filters were washed twice with 5 ml of cold buffer, dried, extracted with 10 ml Aquasol (23°C for 10 min with vigorous shaking) and counted in a Beckman scintillation counter with 40% efficiency. The pH of the incubation medium was unperturbed by any of the ionic additions. Anions were all added as ammonium salts, except for succinate and nitrite which were sodium salts. Nonspecific binding measured in the presence of $1 \mu\text{M}$ unlabelled diazepam was unaffected by the ions, even at the highest concentrations, and was $7.5 \pm 2\%$ of the maximal binding. Each point indicates the mean of three determinations. Triplicate control tubes were placed at the beginning and end of each dose-response curve to detect any instability. For clarity, data for representative inactive anions have been plotted (compare with Table 1). All inactive anions produced either no significant effect on ^3H -diazepam binding or, more often, a small ($<20\%$) inhibition of binding.

Table 1 Effects of anions on ^3H -diazepam binding: correlation with effects on permeability

Anion	Enhancement of binding	Permeability (Eccles ¹⁵)	ED ₅₀ (mM)	E _{max} * (% control binding)
Iodide	+	+	2.8	160
Bromide	+	+	9.0	175–180
Chloride	+	+	13.0	142
Nitrite	+	+	20.0	148
Thiocyanate	+	+		126
Perchlorate	+/-	+		
Chlorate	-	+		
Azide	-	+		
Formate	-	+		
Fluoride	-	-		
Oxalate	-	-		
Succinate	-	-		
Acetate	-	-		
Sulphate	-	-		
Lactate	-	-		
Propionate	-	-		
Maleate	-	-		
Phosphate	-	-		

There were no significant effects of choice of anion, for example, Na^+ , K^+ , NH_4^+ , in the case of bromide or iodide.

* E_{max}, maximal binding relative to control.

closely associated with a chloride ion channel which is also closely linked to the GABA-recognition site.

A crude membrane preparation from rat brain, obtained and incubated in an organic buffer (Tris-maleate), was assayed for ^3H -diazepam binding in the presence of increasing concentrations of some anions; a dose-dependent enhancement of specific binding was demonstrated (Fig. 1). Of 18 anions tested, only five proved to be clearly active—iodide, bromide, chloride, thiocyanate and nitrite. Inactive anions included organic, monovalent (formate, acetate, lactate, propionate) and divalent (oxalate, succinate, maleate), as well as inorganic, monovalent (fluoride, azide, chlorate) and divalent (sulphate). Perchlorate was active only at 25 mM. Since the dose-response curve for each of these anions differs both in regard to ED₅₀ and maximal effect, it is difficult to assess a single sequence of potency. In terms of ED₅₀, iodide is the most active (2.8 mM). The maximal effect was observed with 50 mM bromide, which increases the specific binding 70% over the control. For these two anions, significant enhancement of binding was observed with concentrations as low as 1 mM. Further increase in concentration of active ions resulted in a decrease of the binding, perhaps due to nonspecific effects, consistent with the observation that most of the inactive anions tested were also slightly inhibitory at higher concentrations. The observations were independent of the cation—sodium, potassium and ammonium salts of bromide showed no statistically significant differences in their effectiveness. The effect of anions was to alter specific binding; there were no changes in nonspecific binding values for any anion tested, even at massive concentrations.

The magnitude of the anion effect is strongly dependent on temperature, being maximal at 0 °C and completely abolished at 37 °C. To characterise the anion-dependent increase of binding further, dose-response curves of the inhibition of ^3H -diazepam binding by increasing concentrations of unlabelled ligand in the presence or absence of iodide (25 mM) were obtained, and these data used to construct a Scatchard plot (Fig. 2). The enhanced binding was due to an increase in the affinity constant, while the number of binding sites was unchanged. Pooling data from three experiments, we obtained a mean K_d value of $3.57 (\pm 0.3 \text{ s.e.m.}) \times 10^{-9} \text{ M}$ in the absence of ion, which decreased to $2.4 (\pm 0.13) \times 10^{-9} \text{ M}$ in the presence of 25 mM iodide. The mean binding capacity was $54 (\pm 9.7) \text{ fmol mg}^{-1}$ (original weight) for the control and $60 (\pm 10.5) \text{ fmol mg}^{-1}$ with added iodide. This latter difference was not statistically significant. The

change in the apparent equilibrium constant is attributable to a change in the dissociation rate constant without alterations in the association rate constant: the $t_{1/2}$ for dissociation is 3.5 min (± 0.52) for the control and 6.2 (± 0.91) in the presence of 25 mM iodide (Fig. 2, inset). Association curves with or without ionic additions showed no appreciable difference in the association rate constant, but only in the plateau value due to the change in K .

The selectivity of the effective anions on binding is in excellent agreement with their selectivity for penetrating the activated inhibitory postsynaptic membrane of cat motoneurons measured electrophysiologically¹⁷ (Table 1). Every anion which increased diazepam binding has been reported to convert the inhibitory postsynaptic potential into the depolarising direction. Of particular note is the complete inactivity of fluoride, both in the binding assay and the electrophysiological studies, in spite of the high potency of the other halides. Chlorate, azide and formate, however, failed to enhance ^3H -diazepam binding, but seem to pass through the chloride channel¹⁵. A weak anion effect might easily be masked by the nonspecific inhibitory effect (due to increased ionic strength) that we observed with all the anions tested (at high concentrations for the active ion and at lower concentrations for the inactive ions). For example, perchlorate showed an enhancement of binding at only one concentration (25 mM). Alternative explanations for the anion effect which invoke nonspecific effects (ionic strength, chaotropic effect or effects on water structure in the vicinity of the membrane) are relatively unlikely. Chlorate, for instance, is a strong chaotropic agent¹⁹ but is inactive in this system.

The failure to find enhancement of ^3H -diazepam binding by anions has been reported²⁰, but those authors used Tris-HCl buffer, which obscures the effect.

The temperature dependence of the effect²⁹ which is reminiscent of the sodium effect on opiate binding²¹, the change

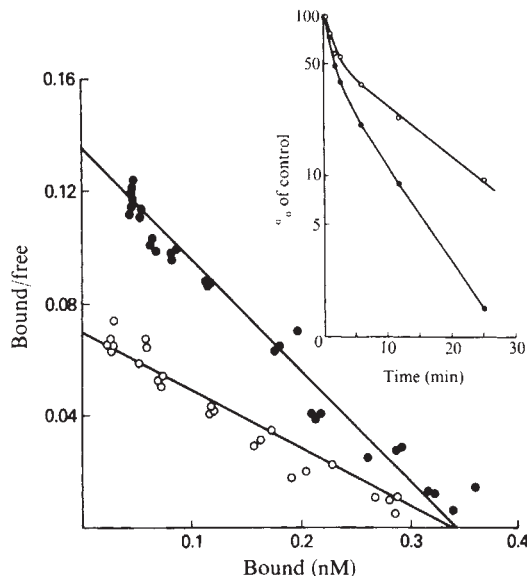


Fig. 2 Scatchard analysis of ^3H -diazepam binding in the presence (●) or absence (○) of 25 mM NH_4I . Assay conditions as described for Fig. 1 with a fixed amount of ^3H -diazepam (0.15 nM) and varying concentrations of unlabelled ligand. Data from three experiments were analysed with a weighted, nonlinear, least squares, curve-fitting program (SCATFIT)^{27,28}. Binding parameters were compared within experiments using a paired analysis. In all three experiments, a major change in affinity was detected, whereas the binding capacity was not significantly affected. The equilibrium constant of association (K) was increased by a factor of $1.39 (\pm 0.04)$ ($P < 0.01$), and the ratio of binding capacities was $1.12 (\pm 0.09)$. Inset shows the dissociation curves in the presence (○) and absence (●) of 25 mM NH_4I . The ratio of the corresponding $t_{1/2}$ values (1.77) is sufficient to explain the difference in the equilibrium constants without a change in the association rate constant.

in affinity due to a change in the dissociation rate constant, and the apparent specificity of the anions involved, all suggest that ions modulate the affinity of the BZ receptor through an allosteric-induced change of conformation. Recent experiments in our laboratory²⁹ have demonstrated that the GABA-induced increase in diazepam binding can be increased markedly and synergistically by including halides in the incubation medium. Since neurophysiological²²⁻²⁴ and binding studies²⁵ already indicate an association of GABA receptor to chloride ion channels, the present data further support the concept that GABA and diazepam receptors are closely associated and may be jointly coupled to a chloride ion conductance mechanism, at least in some regions of the brain.

An interesting speculation about the nature of the endogenous ligand for the diazepam receptor may be made on the basis of analogy with the effects of ions on the opiate, GABA, and α -adrenergic receptors²⁶. In these three systems, antagonist binding is unaffected or enhanced by the ion related to the conductance mechanism, whereas agonist binding is inhibited by the same ion. If this were to represent a general phenomenon, it could be predicted that the endogenous ligand for the 'Valium' receptor would be an 'agonist', while BZs themselves are antagonist compounds; in other words, the endogenous ligand should be an 'anxiety factor'.

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Transplacental effects of chemical mutagens detected by the micronucleus test

EXPOSURE to chromosome-damaging chemicals during prenatal development is particularly hazardous, since stem-cells needed to support tissue maintenance throughout life may then be at risk. Lesions in fetal cells lead to cancer in early life, and in germ-cell lineages, contribute to increased inherited abnormalities. Chemicals can be screened for chromosome-breaking

potential *in vivo* by the micronucleus test¹ which measures frequencies of acentric chromosome fragments². Micronuclei are usually scored in polychromatic erythrocytes from adult rodent bone-marrow. This short-term test is claimed to be as reliable as the more laborious analysis of metaphase chromosomes for chemically-induced visible aberrations^{3,4}. The reactive forms of chromosome-damaging chemicals are often metabolites formed within tissues of exposed individuals. Short-term methods to determine genome-damaging potential must incorporate realistic activating systems if derived risk-estimates are to be meaningful. Although fetal organs contain enzymes which activate mutagenic/carcinogenic chemicals⁵, the micronucleus test has not previously been applied to transplacental exposure. Adult bone marrow has limited activation capacity and short lived mutagens/carcinogens formed in other tissues may not survive in the circulation, so false-negative results can be obtained. Late in gestation, blood cells develop in the fetal liver, alongside hepatocytes which metabolise pre-mutagens/carcinogens to active forms. We show here that transplacental exposure to chemicals which require metabolic activation can cause chromosome breakage, indicated by micronucleus formation, in mouse fetal tissue. The dose dependence of this intrauterine genetic damage can be measured, thus providing a method for quantitative assessment of risks inherent in prenatal exposure to certain classes of carcinogenic and mutagenic chemicals.

The kinetic model presented in Fig. 1 (data from refs 6-9) predicts that transplacental and maternal effects of chromosome-damaging agents could be compared by measuring the incidence of polychromatic erythrocytes containing micronuclei in fetal liver 12-18 h after the effective exposure to the test agent, and in both maternal bone marrow and fetal blood 30 h after the effective exposure. The essential parameters on which this prediction is based are as follows. Optimal expression of chromatid and chromosomal damage occurs if the toxic agent

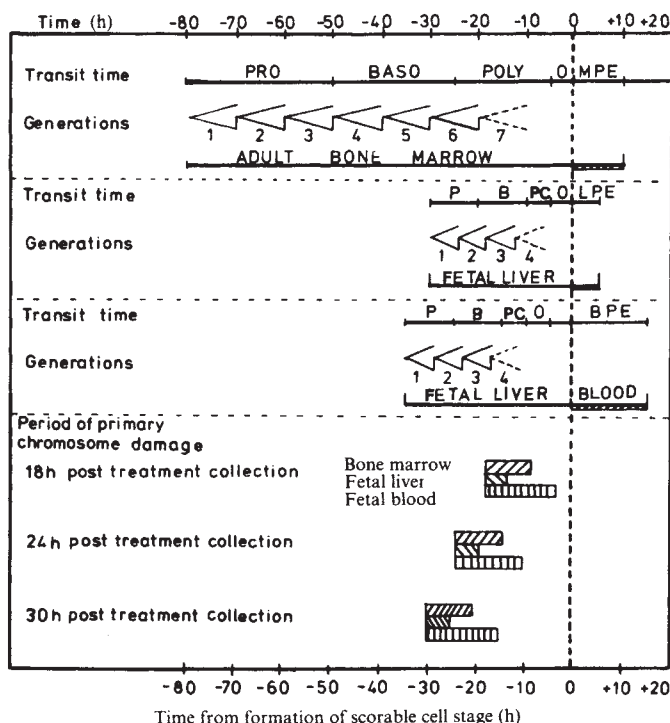


Fig. 1 Kinetic basis for optimal 'expression time' of chromosome lesions giving rise to micronuclei in polychromatic erythrocytes of maternal bone marrow, and fetal liver and peripheral blood, after treatment with chemical mutagens/carcinogens (Pro (P), proerythroblast; Baso (B), basophilic; Poly (PC), polychromatic; O, orthochromatic erythroblasts; MPE, LPE and BPE bone marrow, fetal liver and fetal blood polychromatic erythrocytes, respectively). The dotted line indicating the cell generation within the polychromatic erythroblast class is to show that some of these cells will be post-mitotic.

reaches effective concentrations during the penultimate cell division cycle of erythroblast maturation. Adult bone-marrow erythroblasts undergo six to seven divisions, the last two occupying about 20 h. The post-mitotic cells develop for 10 h until nuclear extrusion is completed and the resulting polychromatic erythrocyte remains in the bone marrow for about 10 h. Fetal liver erythroblasts (after the 16th day of gestation) undergo four divisions, the last two occupying about 12 h. The post-mitotic cells develop for about 5 h until nuclear extrusion is completed, and the polychromatic erythrocyte remains in the fetal liver for about 5 h. Once in the fetal circulation it persists in its early form, with homogeneously distributed cytoplasmic RNA, which gives characteristic staining properties, for about 15 h.

If each target cell received damage leading to formation of a single micronucleus, the maximum incidence of polychromatic erythrocytes containing micronuclei in fetal liver and bone marrow would be ~25%. However, this maximum may not be expressed, as doses causing this degree of damage would be likely to cause other cellular lesions, and interfere with erythroid maturation.

As the daily output of early erythrocytes from the liver is about 60% of the number already in the fetal circulation, the frequency of micronucleated early polychromatic erythrocytes in the blood will be about 60% of the peak in the fetal liver.

These predictions were tested by exposing 15–16-d pregnant Swiss-albino mice to 2 mg per kg mitomycin-C. This substance, produced by the soil microorganism *Streptomyces caespitosus*, is biologically inactive in its natural state but is converted to mono- and bifunctional alkylating agents *in vivo* after reduction to hydroquinone derivatives by NADPH-dependent quinone reductase. The chromosome-damaging potential of mitomycin-C has been established in a variety of plant and animal systems and the agent has been used in the treatment of advanced cancers. The data in Fig. 2 indicate that the predictions of our model are substantially fulfilled. The dose of 2 mg per kg intraperitoneally (i.p.) (~40% of the intravenous LD₅₀) induces

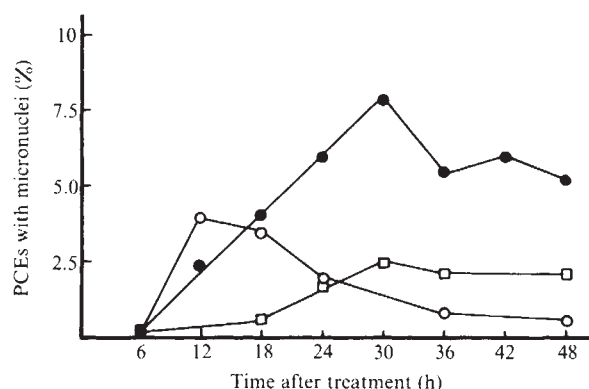


Fig. 2 Time course of incidence of polychromatic erythrocytes (PCEs) containing micronuclei in maternal bone marrow (●), fetal liver (○) and fetal blood (□) after a single i.p. administration of 2 mg per kg mitomycin-C. Data from two experiments. Total of at least four pregnant animals and two fetuses per litter examined for each point. Single cell suspensions of fetal liver and adult femoral bone marrow were prepared in freshly filtered (0.22 μ m pore size) Hank's balanced salt solution +5% fetal calf serum, by repeated pipetting. Slides were prepared on a 'Cytospin' (Shandon) (4×10^4 nucleated cells in 0.2 ml per slide). Blood films were prepared directly from dissected fetuses. Slides were air dried, fixed in absolute methanol, and stained: May Grunwald (Merck) 0.25% in methanol 3 min; May Grunwald as above diluted 1:1 with Sorenson buffer, pH 6.8, 2 min; Giemsa (Merck) 14% v/v in Sorenson buffer, pH 6.7, 10 min. Stained slides were rinsed briefly in buffer followed by distilled water (total 30 s for bone marrow, 45 s for fetal liver and blood). All solutions were filtered through Whatman GFC immediately before use. This protocol maximises the distinction between polychromatic erythrocytes (blue cytoplasm) and mature erythrocytes (yellow-orange cytoplasm) and stains micronuclei red-purple. Air-dried slides were mounted in DePex. A minimum of 1,000 polychromatic erythrocytes were evaluated per slide with a 100 $\times$ objective.

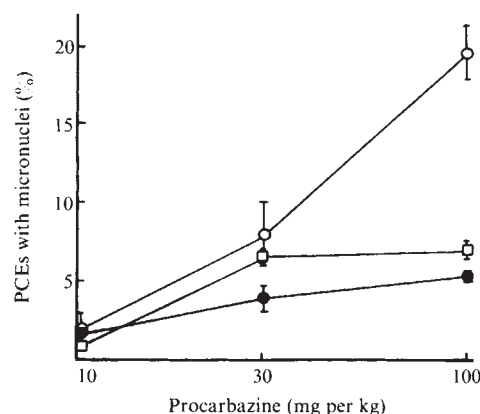


Fig. 3 Dose responses to procabazine. Fetal liver (○), fetal blood (□) and maternal bone marrow (●) were examined for frequency of polychromatic erythrocytes (PCEs) containing micronuclei 12, 18, 24, 30 and 36 h after 10, 30 or 100 mg per kg procabazine had been administered by single i.p. injection. At least three pregnant animals and two fetuses from each of the three litters were examined per point. Data plotted from time giving maximum incidence for each dose and tissue and expressed as mean $\pm$ s.e.

approximately twice the frequency of micronucleated erythrocytes in the adult bone marrow as in the fetal liver. The spontaneous incidence of micronucleated erythrocytes was $0.16 \pm 0.03\%$ in adult bone marrow, $0.40 \pm 0.08\%$ in the fetal liver, and $0.24 \pm 0.09\%$ in the fetal blood.

Similar tests were carried out with procabazine hydrochloride, another anti-neoplastic drug, using single i.p. injections of 100 mg per kg (40% of LD₅₀), 30 mg per kg and 10 mg per kg. This agent is enzymatically converted to biologically active formaldehyde, methyl diazene and methyl radicals, through azo procabazine. Activity of the three available routes (*N*¹ hydroxylation, *N*¹ demethylation or *N*² hydroxylation) may vary between tissues. Although clearly mutagenic to mammalian cells *in vivo*, mutagenicity of procabazine cannot be reliably detected by *Salmonella* or *Saccharomyces* systems^{10, 12}. Figure 3 shows that dose-response curves can be constructed from all three tissues examined. Fetal liver is more damaged by procabazine than adult bone marrow.

These preliminary experiments establish the usefulness of the erythrocyte micronucleus test applied to prenatal mice. The frequency of spontaneous micronucleus formation in the fetal liver is low, and preparations of fetal liver and fetal blood cells for microscopic observation are simply made and scored. We are extending this analysis to a wider range of environmentally significant mutagens and carcinogens, including those which have given negative results in the standard adult erythrocyte micronucleus test.

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Reduction of benzo(a)pyrene mutagenicity by dihydrodiol dehydrogenase

THE enigma of how inert chemicals can exert potent mutagenic, carcinogenic, allergenic and cytotoxic effects has been much debated. It has been learned that such compounds are metabolically converted to chemically reactive species¹. In the case of aromatic or olefinic compounds, monooxygenases located in the membranes of the cell can transform these compounds into epoxides²⁻⁵ which by virtue of electrophilic reactivity can bind chemically to cellular macromolecules such as DNA, RNA and proteins, thereby disturbing biochemical control mechanisms and leading to the above mentioned toxic effects. The same membranes in which such epoxides are produced possess an enzyme, epoxide hydratase, which is capable of inactivating epoxides²⁻⁴. However, with polycyclic hydrocarbons the situation is more complicated. If the epoxide groups are located on a benzo ring opposite a bay region, the resulting dihydrodiols are precursors of even more reactive dihydrodiol epoxides which readily bind to DNA⁶, are often strongly mutagenic⁷⁻¹⁰ and may be mainly responsible for carcinogenic effects of some polycyclic hydrocarbons¹¹. Because, with the environmental carcinogen benzo(a)pyrene, epoxide hydratase was unable to inactivate these dihydrodiol epoxides^{12,13}, we have investigated the ability of a dihydrodiol dehydrogenase¹⁴⁻¹⁶ to sequester the precursor dihydrodiols and thereby afford protection. To this end we have used bacterial mutagenesis¹⁷ as the biological endpoint to investigate the metabolic activation of benzo(a)pyrene by microsomal monooxygenase. Unfortunately, the dihydrodiol dehydrogenase requires the same pyridine nucleotide cofactor as the monooxygenases, so that simple removal or addition of cofactors could not be used to study the role of this enzyme. Therefore purification of the enzyme was necessary. We show here that in metabolic situations where epoxide hydratase cannot protect against benzo(a)pyrene metabolite-evoked mutagenicity, pure dihydrodiol dehydrogenase reduces it. This demonstrates another factor which could contribute to differences in susceptibility to polycyclic hydrocarbon-mediated toxicity between various animal species, strains, sexes, organs and developmental stages, and to differences in potency of polycyclic compounds. This enzyme also provides an additional

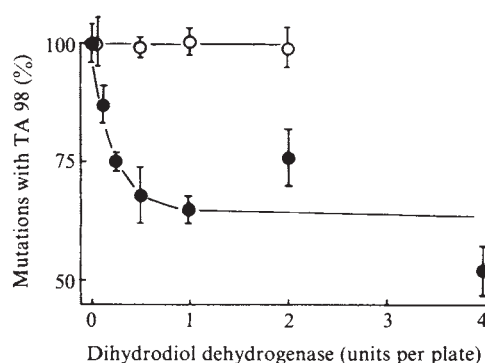


Fig. 1 Effect of dihydrodiol dehydrogenase on the mutagenicity of benzo(a)pyrene activated by liver microsomes from control (○) or 3-methylcholanthrene-treated (●) mice. Benzo(a)pyrene (5 µg, dissolved in 10 µl dimethylsulphoxide), 500 µl of a microsomal metabolising system (1 mg liver microsomal protein from adult male C3H mice, 2 mM NADP⁺, 2 mM NADPH, 8 mM MgCl₂, 80 mM KCl, 50 mM Na phosphate, pH 7.4) 100 µl *his*⁻ bacteria (1.7 × 10⁸ *S. typhimurium* TA 98), various amounts of pure dihydrodiol dehydrogenase (in 100 µl 150 mM KCl) and 2,000 µl histidine-poor top agar (0.55% agar, 0.55% NaCl, 50 µM histidine, 50 µM biotin, 25 mM Na phosphate, pH 7.4, 45 °C) were mixed in a test tube and poured onto a minimal agar plate. After incubation for 3 d in the dark, colonies (*his*⁺ revertants) were counted. Values are means ± s.e.m. of 3–10 parallel incubations.

tool for studying the molecular mechanisms of chemical carcinogenesis.

Dihydrodiol dehydrogenase activity was measured by following the production of NADPH spectrophotometrically at 340 nm. The incubation, which was carried out at 37 °C, consisted of *trans*-1,2-dihydroxy-1,2-dihydrobenzene (1.8 mM), NADP⁺ (2.2 mM), glycine buffer (50 mM, pH 9.0) and the enzyme sample. One unit of enzyme activity was defined as the amount of enzyme which produced 1 µmol NADPH per min in these conditions. The 100,000g supernatant of liver homogenate of adult male Sprague-Dawley rats contained about 0.01 units dihydrodiol dehydrogenase per mg protein. The enzyme was purified about 500-fold with respect to rat liver 100,000g supernatant by a method which included ammonium sulphate precipitation, DEAE-cellulose chromatography, interfacial salting-in and gel filtration through Sephadex G-100. The preparation showed a single polypeptide band when analysed by SDS-gel electrophoresis and sedimented as a single symmetrical peak in the analytical ultracentrifuge in the absence of SDS.

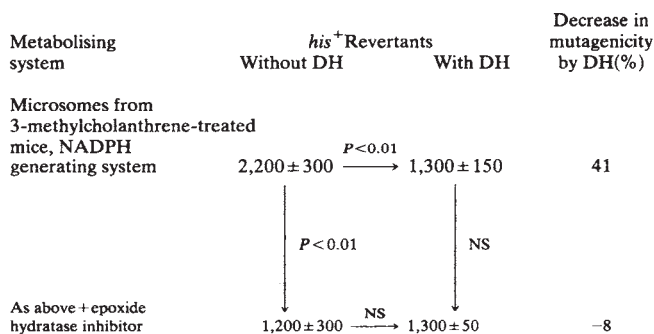


Fig. 2 Effect of dihydrodiol dehydrogenase on the mutagenicity of benzo(a)pyrene with or without simultaneous inhibition of epoxide hydratase. Adult male C3H mice received a single intraperitoneal injection of 3-methylcholanthrene (80 mg per kg body weight, dissolved in sunflower oil) 40 h before death. The livers were homogenised in three volumes 0.15 M KCl buffered with 10 mM Na phosphate, pH 7.4, the homogenate was centrifuged for 10 min at 10,000g, and the resulting supernatant fraction centrifuged for 1 h at 100,000g to give a microsomal pellet. Benzo(a)pyrene (5 µg, dissolved in 10 µl dimethyl sulphoxide), 500 µl of a microsomal metabolising system (1 mg microsomal protein), 1 unit glucose-6-phosphate dehydrogenase, 36 mM NADP⁺, 15 mM glucose-6-phosphate, 8 mM MgCl₂, 80 mM KCl, 50 mM Na phosphate, pH 7.4), where indicated an epoxide hydratase inhibitor²⁶ (0.3 µl 1,1,1-trichloropropene 2,3-oxide) and/or 0.25 units dihydrodiol dehydrogenase (DH, dissolved in 100 µl 0.15 M KCl), 100 µl *his*⁻ bacteria (1.7 × 10⁸ *S. typhimurium* TA 98) and 2,000 µl histidine-poor top agar (0.55% agar, 0.55% NaCl, 50 µM histidine, 50 µM biotin, 25 mM Na phosphate, pH 7.4, 45 °C) were mixed in a test tube and poured onto a minimal agar plate. After incubation for 3 d in the dark, colonies (*his*⁺ revertants) were counted. The number of revertant colonies in the absence of benzo(a)pyrene (38 colonies) was subtracted. Values represent means ± s.d. of 3 parallel incubations. Statistical significance was calculated with the Student's *t*-test; NS, not significant.

Refiltration through Sephadex G-100 resulted in a single protein peak of uniform specific activity. All these findings suggest that the preparations were homogeneous.

The effect of this pure enzyme on the mutagenicity of benzo(a)pyrene was studied in the microsome/*Salmonella* test¹⁷. In this test two metabolic pathways mainly contribute to the mutagenicity of benzo(a)pyrene¹²: (1) activation to benzo(a)pyrene-4,5-oxide^{12,18} is predominant when liver microsomes from control animals are used; (2) dihydrodiol epoxides are mainly responsible for the mutagenicity when low doses of benzo(a)pyrene are activated by microsomes from animals in which cytochrome P448 is induced (by treatment with polycyclic hydrocarbons or with Aroclor 1254)¹². In this situation simple epoxides again become the major mutagenic metabolites if epoxide hydratase is inhibited, or at benzo(a)pyrene concentrations which are high enough to inhibit

oxidation of the dihydrodiols. The *Salmonella typhimurium* strains TA 98 or TA 100 are highly susceptible to both groups of mutagenic metabolites, whereas the strain TA 1537 is much more susceptible to the 4,5-oxide than to the dihydrodiol epoxides¹².

Figure 1 shows the effect of dihydrodiol dehydrogenase in these two conditions. When benzo(a)pyrene was activated by liver microsomes from 3-methylcholanthrene-treated mice, addition of pure dihydrodiol dehydrogenase clearly reduced the mutagenicity. The effect increased with the dose of dihydrodiol dehydrogenase up to 0.5 units per plate; further addition of dihydrodiol dehydrogenase had no significant effect. Thus, maximal reduction of mutagenicity was achieved using about 15 times more enzyme than that present in the amount of liver equivalent to the microsomes used for activation. Similar, and even greater, differences in activities of foreign compound-metabolising enzymes are encountered between various animal species^{19,20} or after exposure to foreign compounds with inducing properties^{21,22}.

The percentage of the benzo(a)pyrene mutagenicity which could be removed by dihydrodiol dehydrogenase depended on the bacterial strain. With TA 98, it was 30–60%, and with TA 1537, as expected from the lower sensitivity of this strain for benzo(a)pyrene dihydrodiol epoxides, it was smaller, 15–25% (data not shown). In contrast to these experiments with microsomes from 3-methylcholanthrene-treated mice, no effect of dihydrodiol dehydrogenase on the mutagenicity of benzo(a)pyrene was found when microsomes from control mice were used (Fig. 1). This is readily understandable, as control mouse liver microsomes activate benzo(a)pyrene mainly to benzo(a)pyrene-4,5-oxide rather than to dihydrodiol epoxides^{12,18}. This is the metabolic situation where epoxide hydratase markedly reduced the mutagenicity^{12,23}. Similarly, with 3-methylcholanthrene-induced microsomes in which the formation of dihydrodiols was prevented by inhibition of epoxide hydratase (Fig. 2), dihydrodiol dehydrogenase failed to reduce the mutagenicity of benzo(a)pyrene. The dihydrodiol dehydrogenase-resistant mutagenicity is probably due to simple epoxides^{13,18,24,25}, phenols^{24,25} and metabolites of phenols¹³ which are generated by the mammalian activating system.

Thus we conclude (1) that dihydrodiol dehydrogenase can protect against mutagenic effects of benzo(a)pyrene, but (2) that not all the mutagenic metabolites of benzo(a)pyrene can be removed by dihydrodiol dehydrogenase. (3) Probably due to the combination of a relatively efficient inactivation of other active metabolites of benzo(a)pyrene and the high inherent biological activity of dihydrodiol bay-region epoxides, the latter seem to play an especially important part in mutagenicity, DNA binding, cell transformation and tumour formation^{8–11}. Therefore, the discovery that dihydrodiol dehydrogenase is capable of reducing the mutagenicity mediated by the dihydrodiol–epoxide pathway opens an intriguing perspective for the entire field of polycyclic hydrocarbon-evoked mutagenicity, carcinogenicity and cytotoxicity.

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Conformation of Moloney murine leukaemia proviral sequences in chromatin from leukaemic and nonleukaemic cells

THE structure of transcriptionally active and inert regions of eukaryotic chromatin can be differentiated on the basis of their sensitivity towards nuclease digestion. Whereas DNase I preferentially digests transcriptionally active genes into fragments too small to form stable hybrids, micrococcal nuclease shows no preferential digestion of specific nuclear DNA sequences^{1–6}. We report here experiments in which the structure of Moloney murine leukaemia proviral DNA sequences in chromatin of target cells expressing the viral genome and of non-target cells in which the same sequences are repressed were examined by digestion with DNase I and micrococcal nuclease. It is shown that the Moloney murine leukaemia virus (M-MuLV) genomes in target and non-target cells of BALB/Mo mice are integrated into the host cell genome and organised in nucleosome-like structures. The difference in transcriptional activity of viral genomes in target and non-target cells is reflected by a difference in DNase I sensitivity. A partial resistance to DNase I of M-MuLV sequences in target cells suggests that the multiple viral genome copies in these cells are integrated at different sites and have different transcriptional activity.

Retroviruses provide excellent model systems for studying the structure of eukaryotic chromatin. As endogenous viruses they are normal constituents of the genomes of a wide variety of animal species. Alternatively, they can integrate into the genome of their host organisms after exogenous infection where they serve as template for the transcription of viral mRNA and progeny genome RNA (refs 7, 8). The expression of the integrated retroviral genome is subject to control by genetic factors of the host⁹ and is linked to the differentiated state of the infected cell¹⁰. Thus, these viruses offer the unique opportunity for studying the structural organisation of well defined DNA sequences in various states of expression.

The experiments described here were carried out with nuclei isolated from different organs of BALB/Mo mice and therefore reflect the state of proviral DNA sequences in the animal. BALB/Mo mice were derived from an animal infected at the four- to eight-cell preimplantation stage with M-MuLV. They carry the M-MuLV genome integrated into the DNA of all organs and transmit it like an endogenous virus^{11,12}. The genetically transmitted viral structural gene, the *Mov-1* locus, has recently been assigned to mouse chromosome 6 (ref. 13). BALB/Mo mice develop a specific, thymus-derived leukaemia. Expression of the M-MuLV genome during leukaemogenesis is restricted to lymphatic target tissues (spleen, thymus) and is

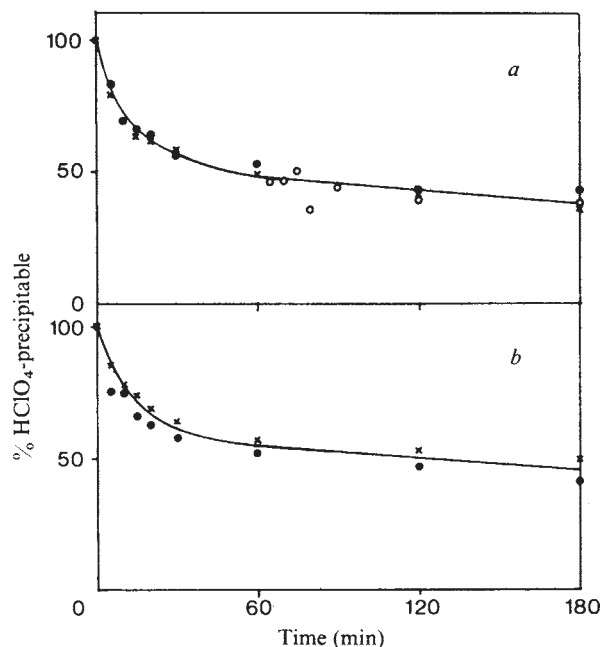


Fig. 1 Kinetics of digestion of mouse liver and spleen chromatin with *a*, DNase I, and *b*, micrococcal nuclease. Nuclei were isolated from homogenised mouse tissues by centrifugation through 2.2 M sucrose and digested at 37 °C at a nuclear DNA concentration of 1 mg ml⁻¹. Enzyme concentrations were 10 units ml⁻¹. The percentage of DNA remaining HClO₄-precipitable was determined by precipitation with cold 7% HClO₄ and subsequent measurement of the absorbance at 260 nm of the acid-soluble and insoluble fraction. ●, Spleen nuclei; ×, liver nuclei; ○, fresh enzyme was added after 1 h.

correlated with a somatic amplification of viral DNA sequences^{11,14}. In contrast, the viral genome remains repressed throughout the life of the animals and no amplification of viral sequences occurs in non-target organs (such as liver or brain)¹⁴.

We have carried out DNase I and micrococcal nuclease digestions of chromatin from target (spleen) and non-target (liver) cells of homozygous¹² leukaemic BALB/Mo mice. The conditions of digestion with both enzymes were chosen to render approximately 50–60% of the DNA HClO₄-soluble within 60 min. Further incubation or the addition of fresh enzyme did not significantly increase the HClO₄-soluble fraction, indicating that the undigested fractions were resistant to the action of the enzymes (Fig. 1). These results confirm the findings of others¹. After different periods of DNase digestion, aliquots were withdrawn and the DNA was purified and analysed for M-MuLV-specific sequences by a quantitative complementary DNA excess hybridisation using an M-MuLV-specific cDNA which does not recognise endogenous virus sequences^{12,15}. As shown in Fig. 2*a*, M-MuLV-specific sequences were preferentially digested by DNase I in spleen cells of homozygous leukaemic BALB/Mo mice: whereas undigested DNA from these cells contained three to four M-MuLV genome copies per haploid mouse genome equivalent, this number was reduced to approximately two copies after digestion with DNase I for only 10–15 min. This was not observed in liver cells from the same animals: liver cells contained one viral genome copy per haploid mouse genome, which was not preferentially digestible by DNase I (Fig. 2*a*).

The preferential digestibility by DNase I of M-MuLV-specific sequences in chromatin from spleen but not from liver cells of homozygous BALB/Mo mice agrees with the finding that the viral genome, although genetically transmitted and present in every cell of these mice, is expressed only in 'target' organs such as spleen and thymus, whereas it stays repressed in 'non-target' organs such as liver and brain¹⁴. However, as shown in Fig. 2*a*, in spleen cells of homozygous, leukaemic mice, in which an

amplification of the viral genome from initially one to three to four copies per haploid genome had taken place, only two of the copies were sensitive to DNase I, whereas the remaining one to two copies showed the same relative resistance towards DNase I as the bulk of the host DNA sequences. In analogy to the findings of others^{1–6}, we assume that the DNase I-sensitive sequences are transcriptionally active, whereas the resistant ones are inactive. This assumption is supported by the preliminary finding that in young homozygous BALB/Mo mice which contain only one M-MuLV genome copy in their target cells¹⁴, this single copy is sensitive to DNase I digestion.

The partial resistance of M-MuLV sequences to DNase I in spleen cells of homozygous, leukaemic BALB/Mo mice (Fig. 2*a*) therefore suggests a difference in the transcriptional activity of the various viral genome copies in leukaemic tissues. On the basis of these data, however, we cannot exclude a heterogeneity of the cell population in the sense that in some cells all M-MuLV sequences are DNase I-sensitive and in others all are resistant. Furthermore, as the complexity of the cDNA probe used in the hybridisation analysis has not been accurately determined¹² and nothing is known about the molecular organisation of the various M-MuLV genome copies integrated in leukaemic cells,

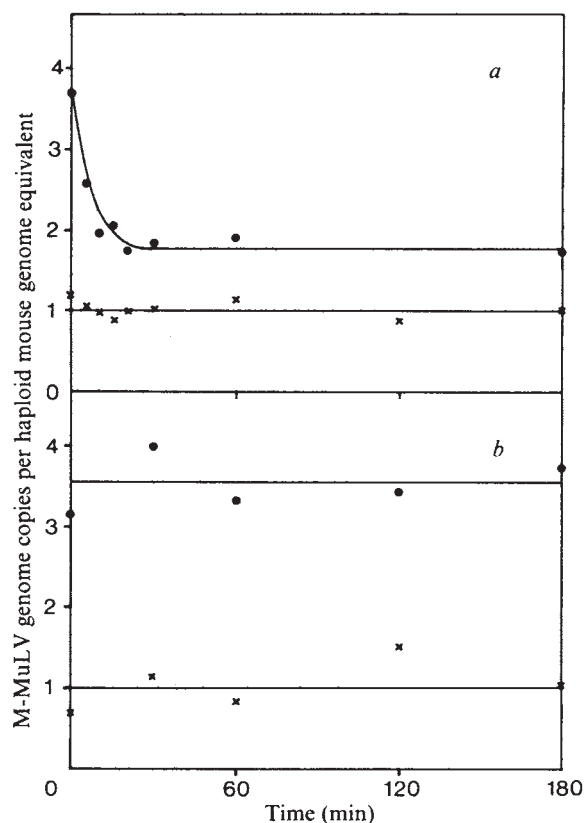


Fig. 2 Determination of M-MuLV-specific sequences in DNase-digested chromatin by molecular hybridisation. Nuclei from spleens (●) and livers (×) of homozygous leukaemic BALB/Mo mice were isolated and digested with *a*, DNase I, and *b*, micrococcal nuclease, as described in Fig. 1. After the times indicated, undigested DNA was extracted from the DNase-treated nuclei, purified by CsCl-ethidium bromide equilibrium centrifugation and boiled in alkali as described elsewhere^{11,12}. The concentration of M-MuLV-specific DNA sequences was determined in each DNA preparation in a cDNA excess hybridisation assay using an M-MuLV-specific cDNA probe essentially as described elsewhere^{12,15}. M-MuLV genome copy numbers were calculated by comparison with standard mouse DNAs with known contents of viral sequences (homozygous and heterozygous BALB/Mo embryo DNAs containing 1 and 0.5 M-MuLV copies per haploid mouse genome equivalent, respectively¹²). Thus, the ordinate represents M-MuLV genome copies per weight of undigested DNA expressed as haploid mouse genome equivalents.

Table 1 Hirt fractionation

DNA source	M-MuLV genome copies per haploid mouse genome	% Of total DNA in		% Of M-MuLV-specific DNA in
		supernatant	Precipitate	
Spleen	3.4	2.5	2.4	97.6
Liver	1.4	3.8	4.5	95.5
SV40	—	89.5	—	—

Mouse tissues were homogenised in a loose Teflon homogeniser in phosphate-buffered saline (PBS) (liver: 5 ml PBS per g; spleen: 20 ml PBS per g). The homogenate was diluted with 6 volumes of 10 mM Tris-HCl, pH 7.6, 10 mM EDTA, and the high molecular weight DNA was precipitated with 1% SDS and 1 M NaCl as described by Hirt¹⁶. The precipitates were centrifuged (30 min, 27,000 g, 4°C), DNA was extracted and purified from the supernatant and precipitate fractions, and M-MuLV-specific sequences were determined by molecular hybridisation^{12,15}. In a control experiment, radioactively labelled SV40 DNA was added to a spleen homogenate to determine the degree of trapping and co-precipitation of free viral DNA with the Hirt precipitate.

we cannot determine whether parts or all of the viral genomes are in the sensitive configuration.

When similar experiments were carried out with micrococcal nuclease, we found that this enzyme did not preferentially digest viral sequences, in either target or non-target tissues, of leukaemic BALB/Mo mice (Fig. 2b): the relative concentration of viral sequences (three to four copies per haploid genome in spleen and one copy in liver cell chromatin) was constant throughout the course of digestion. This was observed reproducibly in organs from BALB/Mo mice of different age as well as in M-MuLV-producing fibroblasts *in vitro* (data not shown). This result indicates that the M-MuLV genomes in target and non-target organs of BALB/Mo mice, including the copies that are amplified during leukaemogenesis, are organised in similar nucleosomal structures to those of the host cell DNA, and suggests that the multiple viral genome copies in tumour cells are integrated into the host cell genome and not present as free proviral DNA.

To determine directly whether unintegrated proviral DNA contributed to the DNase I-sensitive fraction of the M-MuLV-specific sequences in the experiments described above, we fractionated the DNA from spleen and liver cells of leukaemic homozygous BALB/Mo mice by the Hirt procedure¹⁶ and tested the supernatant and precipitate fractions for M-MuLV-specific sequences by molecular hybridisation. As shown in Table 1, only 3–4% of the total DNA from mouse liver or spleen cells was not precipitated by this procedure. In contrast, when radioactively labelled SV40 DNA was added before the fractionation, almost 90% of the radioactivity was found in the supernatant, eliminating the possibility that unintegrated proviral DNA was trapped and co-precipitated with the cellular DNA. When the Hirt supernatant and precipitate fractions were analysed for M-MuLV-specific sequences, we found that 96% in liver chromatin and 98% in spleen chromatin of the virus-specific DNA was precipitated with the high molecular weight cellular DNA, showing that virtually all the viral sequences are in an integrated state and that only very little, if any, free proviral DNA is present. A similar conclusion was reached by Berns *et al.* (personal communication) on the basis of restriction enzyme analysis of DNA from liver and leukaemic cells from BALB/Mo mice which indicate that the somatic amplification of M-MuLV involves new and independent integration sites.

We have shown here that M-MuLV-specific sequences in chromatin from target and non-target organs of BALB/Mo mice have a different conformation as reflected by a difference in DNase I sensitivity. This confirms that changes in transcriptional activity of eukaryotic genes correlate with conformational changes of the respective chromatin regions^{1–6}. In M-MuLV and AKR virus-induced leukaemia of mice, leukaemogenesis is accompanied by a somatic amplification of virus-specific sequences^{11,14,15}. The studies reported here have shown that the multiple viral genome copies in leukaemic cells are integrated into the host cell genome and that the integrated viral sequences are organised in nucleosomal structures. The partial resistance

to DNase I of M-MuLV-specific sequences in leukaemic cells (Fig. 2a) agrees with the hypothesis that the viral genome copies integrated at different integration sites have different transcriptional activity. Dino and Penhoet¹⁷ have recently come to a similar conclusion by measuring intracellular concentrations of viral RNA in murine leukaemia virus and sarcoma virus-infected cells. It will be of interest to characterise further the various integrated M-MuLV genome copies in leukaemic cells and to correlate the different integration sites to transcriptional activity and leukaemic transformation.

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Simian virus 40 specific proteins on surface of HeLa cells infected with adenovirus 2–SV40 hybrid virus Ad2⁺ND2

CELLS infected or transformed by simian virus 40 (SV40) express SV40 tumour-specific transplantation antigen (TSTA)^{1,2}. The mechanism of tumour rejection mediated by this antigen is not yet understood, but our knowledge about transplantation rejection suggests that TSTA should be located on the cell surface³. SV40 TSTA has a close molecular relationship to SV40 T-antigen^{4,5}, which is a nuclear antigen, and until now, SV40 specific proteins have not been demonstrated unequivocally in the plasma membranes or on the cell surfaces of SV40 infected or transformed cells. In contrast, in cells infected by the nondefective adenovirus 2–SV40 hybrid virus Ad2⁺ND2, a virus known to induce SV40 TSTA⁶ the SV40-specific proteins (MW 56,000 (56K) and 42,000 (42K)) are stably incorporated into the plasma membranes⁷. These proteins are coded for—at least in part—by the SV40 portion of the hybrid virus genome and correspond to the C-terminal half of SV40 T-antigen⁸. Furthermore, these proteins are immunoprecipitable from extracts of Ad2⁺ND2-infected cells using serum from SV40 tumour-bearing hamsters⁷. Analysis of Ad2⁺ND2-infected HeLa cells by immunofluorescence microscopy for cell surface fluorescence using hamster SV40 tumour serum, however, failed to give a positive result (W.D., unpublished). A possible

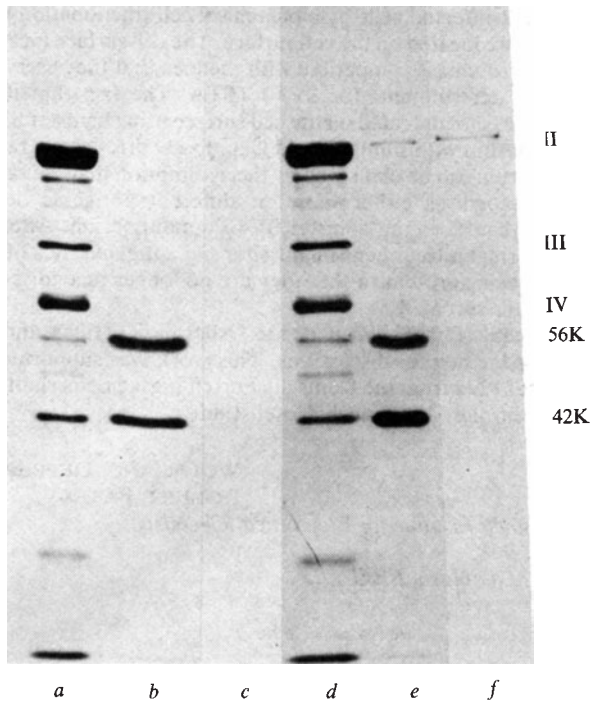


Fig. 1 Immunoprecipitation of SV40 specific proteins from NP40 cytoplasmic extracts of Ad2⁺ND2-infected HeLa cells using hamster SV40 tumour serum or rabbit anti-T serum. HeLa cells grown in suspension were infected with Ad2⁺ND2 and labelled from 40–41 h post-infection with ³⁵S-methionine (20 μ Ci ml⁻¹). NP40 cytoplasmic extract was prepared by lysing the cells with RSB¹⁵ containing 0.5% NP40, and removal of the nuclei by centrifugation (800g for 5 min). Aliquots of 300 μ l of the NP40 cytoplasmic extract were incubated with 30 μ l of serum for 1 h at 0°C and 150 μ l pre-swelled protein A-Sepharose were added per assay and the reaction mixture was incubated for a further 30 min at 0°C. The protein A-Sepharose containing the immune complexes was removed by centrifugation. The protein A-Sepharose was washed extensively with isotonic Tris buffer, pH 7.2, containing 0.2% NP40. Then the immunoglobulins were eluted from the protein A-Sepharose with 500 μ l of 50 mM NH₄HCO₃, containing 1% SDS. The eluates were lyophilised and samples of the NP40 cytoplasmic extract (original reaction mixture), and the eluates were processed for SDS-PAGE analysis as described elsewhere¹⁶. Samples containing ~15 μ g of protein were run on a 8.5% SDS-polyacrylamide gel and radioactive polypeptides were visualised by fluorography as described by Bonner and Laskey¹⁷. The sample order is: a, Ad2⁺ND2 cytoplasmic extract; b, same, eluate after immunoprecipitation with hamster SV40-tumour serum; c, same, eluate after immunoprecipitation with hamster non-immune serum; d, Ad2⁺ND2 cytoplasmic extract; e, same, eluate after immunoprecipitation with rabbit anti-T serum; f, same, eluate after immunoprecipitation with rabbit non-immune serum.

explanation is that upon location on the cell surface the structure of these proteins is altered so that they are no longer recognised by the tumour sera. These sera are probably directed against native T-antigen released from the nuclei of necrotic tumour material. We therefore decided to prepare antisera against SDS-denatured T-antigen which are directed against the primary structure of T-antigen rather than against its native conformation. We report here that SV40-specific proteins can be visualised on the cell surface of Ad2⁺ND2-infected HeLa cells by immunofluorescence microscopy using a rabbit anti-T serum, prepared against purified SDS-denatured T-antigen.

T-antigen was prepared by preparative immunoprecipitation of cell extracts of the hamster tumour line H 65/90 B (originally obtained from Dr V. Defendi) according to the method of Schwyzer⁹ using hamster SV40 tumour serum and protein A-Sepharose. T-antigen was then further purified by preparative SDS-polyacrylamide gel electrophoresis (SDS-PAGE) and electrophoretic elution of the T-antigen band. The electrophoretically pure T-antigen preparation was used for immunisation of a rabbit. The method of preparation of purified T-antigen and the immunisation procedure will be described elsewhere (W.D. and R.P., in preparation). Rabbit anti-T sera have been prepared in similar ways previously^{10,11}. Hamster

SV40 tumour serum (from Dr R. Henning) and the rabbit anti-T serum were first characterised by determination of their T- and U-antibody titres on SV40-transformed mouse cells (clone SV101), on heat-treated SV101 cells¹² and on Ad2⁺ND2-infected HeLa cells¹³, respectively.

Table 1 shows that both sera exhibited similar T-antibody titres on SV101 cells and similar U-antibody titres both on heat-treated SV101 cells and on Ad2⁺ND2-infected HeLa cells.

The specificity of both sera was further assessed by immunoprecipitation of extracts from Ad2⁺ND2-infected cells and analysis of cell extracts and immunoprecipitates by SDS-PAGE. Figure 1 demonstrates that both sera specifically precipitated the SV40-specific 56K and 42K proteins.

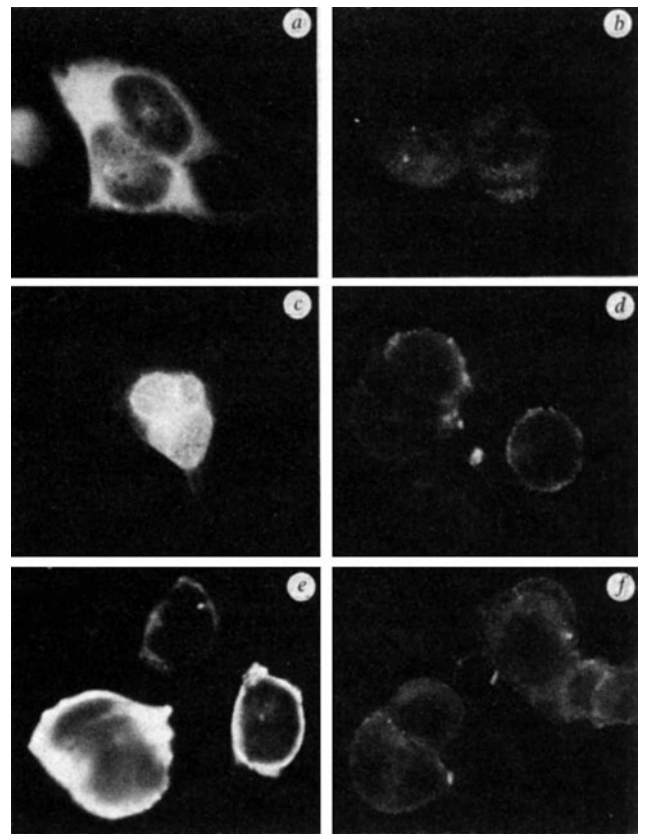


Fig. 2 Visualisation of SV40 specific proteins within and on the cell surface of Ad2⁺ND2-infected HeLa cells by immunofluorescence microscopy. HeLa cells grown on coverslips (12 mm) were infected with Ad2⁺ND2 (a, c, d, e) or Ad2 (b, f) and processed for immunofluorescence microscopy 40 h post-infection as described in Table 1 legend. Cells in panels a, b and c were fixed by methanol-acetone treatment as described in Table 1 legend, cells in panels d, e and f were fixed for cell surface staining by treatment for 5 min with 3.7% formalin in PBS at 0°C followed by air drying. Cells in panels a, b, e and f were stained with rabbit anti-T serum, at a 1:100 dilution (panels a, b) or a 1:10 dilution (panels e, f) with PBS. Cells in panels c and d were stained with a rabbit antiserum prepared against purified HeLa cell nuclei. Cells were viewed with a Zeiss microscope equipped with epifluorescent illumination. Note that the uninfected cell in (a) (lower right) does not exhibit fluorescence staining. Cells in (e) are located in different focal planes. The cell in the middle right area is focused at the cell periphery, the focal plane of the island of two cells in the lower left being slightly above this. The cell in the upper middle area is either uninfected or in a very early stage of infection. Staining of either Ad2- or Ad2⁺ND2-infected HeLa cells with rabbit non-immune serum did not result in immunofluorescence staining (data not shown). Photographs were taken with a Planapo 63 oil-immersion objective. Exposure times for panels a, c and e were ~30 s, for panels b, d and f ~2.5 min. Magnification in all panels is ~ $\times 366$.

The rabbit anti-T serum described above was then used to analyse Ad2⁺ND2-infected HeLa cells by immunofluorescence microscopy. Methanol-acetone fixation and staining with the rabbit anti-T serum and FITC-labelled sheep anti-rabbit-IgG revealed typical perinuclear or cytoplasmic U-antigen fluorescence¹³ in the Ad2⁺ND2-infected cells (Fig. 2a). This U-antigen

fluorescence was found in about 80% of the cells, indicating that about this proportion of the cells were infected by Ad2⁺ND2. Ad2-infected HeLa cells treated with rabbit anti-T serum, on the other hand, did not exhibit any fluorescence staining (Fig. 2b). Staining of living Ad2⁺ND2-infected HeLa cells for cell surface fluorescence was impractical, because late in infection (~40 h) these cells are fragile and tend to lyse during processing for immunofluorescence microscopy. Analysis of Ad2⁺ND2-infected cells for cell-surface fluorescence, therefore, was performed with formalin-fixed cells¹⁸. This treatment stabilises the cells and is believed not to render the cell membrane permeable to antibody. This was tested by analysing Ad2⁺ND2-infected HeLa cells after methanol-acetone and after formalin fixation with an antiserum prepared against purified HeLa cell nuclei.

Table 1 T and U antibody titres of hamster SV40 tumour pool serum and rabbit anti-T serum

Sera	Antibody titres on:		
	SV101 cells (T-Ag)	Heated SV101 cells (U-Ag)	Ad2 ⁺ ND2-infected HeLa cells (U-Ag)
Hamster pool	3,200	800	600
Rabbit anti-T	1,600	1,600	600

Hamster SV40 tumour pool serum and rabbit anti-T serum were tested by immunofluorescence microscopy for T and U antibodies on SV101 cells and Ad2⁺ND2-infected HeLa cells grown on coverslips (12 mm diameter), respectively. U antibody titres on SV101 cells were determined after heat inactivation of T-antigen¹². SV101 cells grown on coverslips (12 mm) were washed twice with phosphate-buffered saline (PBS)¹⁴ and incubated in a wet chamber at 50°C for 30 min. The cells were then washed twice with PBS and processed for immunofluorescence microscopy. SV101 post-infection cells, heat-treated SV101 cells and Ad2⁺ND2-infected HeLa cells (at ~40 h.p.i.) were fixed by treatment with methanol at -20°C for 5 min, followed by acetone treatment at -20°C for 30 s and air drying. The cells were then incubated for 45 min at 37°C with the first antibody (hamster SV40 tumour pool serum or rabbit anti-T serum) at the serum dilutions indicated. After washing extensively with PBS, the second antibody (FITC-labelled γ -globulin prepared against hamster IgG or FITC-labelled γ -globulin prepared against rabbit IgG, respectively) was added and the cells held for a further 45 min at 37°C. After a second series of washes with PBS the coverslips were mounted with Elvanol on microscope slides. The cells were viewed with a Zeiss microscope equipped with epifluorescent illumination. Antibody titres are given as reciprocal of the serum dilution, at which the staining intensity was 1+ on a scale of 4+ = brilliant, and $\pm$ = very dull. Before use all sera were absorbed either on methanol-fixed 3T3 cells (when tested on SV101 cells) or on methanol-fixed, Ad2-infected HeLa cells (when tested on Ad2⁺ND2-infected HeLa cells).

Figure 2 demonstrates that this serum stained the nuclei in methanol-acetone fixed cells (Fig. 2c), but not in formalin-fixed cells (Fig. 2d). This result demonstrated that after formalin fixation antibody was no longer able to permeate the plasma membrane of the hybrid virus-infected cells. Staining of formalin-fixed Ad2⁺ND2-infected HeLa cells with rabbit anti-T serum resulted in typical cell surface fluorescence of approximately 80% of the cells (Fig. 2e). This indicated that the cell surface fluorescence was associated with the Ad2⁺ND2-infected cells. It was not visible on Ad2-infected HeLa cells (Fig. 2f), or when rabbit non-immune serum was used as first antibody (data not shown).

Staining of Ad2⁺ND2-infected cells for surface fluorescence was positive with the rabbit anti-T serum down to a serum dilution of 1:100. However, when Ad2⁺ND2-infected cells were tested for cell surface fluorescence using the rabbit anti-T serum after extensive absorption on methanol-fixed SV101 cells, a procedure which resulted in an 80% reduction of both the T and U antibody titres in this serum, no cell surface fluorescence could be obtained at a 1:10 dilution of the absorbed serum (data not shown). This result is further evidence for the SV40 specificity of the cell surface staining shown in Fig. 2e.

The cell surface staining of Ad2⁺ND2-infected cells described above provides evidence that at least part of the SV40-specific 56K and 42K proteins located in the plasma membranes of

Ad2⁺ND2-infected cells by biochemical cell fractionation procedures⁷ are located on the cell surface. The cell surface location of these proteins is compatible with the idea that they carry the antigenic determinants for SV40 TSTA. The fact that these proteins are not detected on the cell surface using hamster SV40 tumour serum with similar T and U antibody titres as the rabbit anti-T serum can be explained by the assumption that the rabbit serum recognises either more or different antigenic determinants than does the hamster SV40 tumour serum. Alternatively, formalin treatment might alter the antigenic sites of the SV40-specific protein so that they are no longer recognised by the tumour serum.

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Eukaryotic DNA fragments which act as promoters for a plasmid gene

TRANSCRIPTION is the process that occurs in all organisms whereby the genetic information coded by the nucleotide sequences of DNA is transferred into RNA. This process is initiated by attachment of RNA polymerase to a region of the DNA called the promoter¹. Considerable information has accumulated recently concerning the structure and function of prokaryotic promoters, but little insight has been gained into the characteristics of eukaryotic promoters. We report here on experiments that use an *E. coli* plasmid with an attenuated promoter as a probe for possible eukaryotic promoters.

The *E. coli* plasmid pBRH3B is a 5.2 megadalton derivative of pBR316 (ref. 2) which codes for ampicillin (Ap) and tetracycline (Tc) resistance. It was constructed by inserting a chemically synthesised *EcoRI* linker into the RNA polymerase binding site (that is, the promoter) of the Tc-resistance gene, thereby decreasing the Tc resistance conferred by the plasmid from 75 $\mu\text{g ml}^{-1}$ to 5 $\mu\text{g ml}^{-1}$ Tc (ref. 3). Insertion of a fragment of DNA at the *EcoRI* site completely abolishes the residual activity of the resident Tc promoter, and expression of Tc resistance then becomes dependent on 'foreign' promoters contained within the inserted fragments. The level of Tc resistance thus provides a simple biological assay for the presence and possibly the strength of promoter activity carried on cloned fragments.

Purified DNAs from several prokaryotic and eukaryotic organisms (Table 1) were digested with the restriction endonuclease *EcoRI* and cloned into the *EcoRI* restriction site

Table 1 Frequency of promoter active recombinants, expressed as percentage of total recombinants

DNA source	%
<i>E. coli</i> bacteriophage T4	25.3, 26.2
<i>Escherichia coli</i>	29.3
<i>Bacillus subtilis</i>	25.0
<i>Rhizobium japonicum</i>	9.8
<i>Euglena</i> chloroplast	33.0
<i>Saccharomyces cerevisiae</i>	27.4, 28.4
<i>Tetrahymena thermophila</i>	7.57
<i>Neurospora crassa</i>	4.74, 4.65
<i>Drosophila melanogaster</i>	0.77, 0.84
<i>Megascia scalaris</i>	0.23

Purified DNAs from each of the organisms listed above were digested with *Eco*RI, combined with an equimolar amount of *Eco*RI-digested pBRH3B, and incubated with T4 DNA ligase². The ligation mixture was transformed into the *E. coli* strain RR1 and the cells were grown for three generations (3 h) at 37°C in Luria broth (LB) after which 0.1 ml aliquots were spread on LB plates containing 20 µg per ml Ap. Frequencies of total recombinants and of promoter-active recombinants were obtained for each species of DNA by patching 500–2,000 Ap-resistant colonies onto plates containing 5, 10, 15, and 20 µg per ml Tc. Those which did not grow on 5 µg per ml Tc were scored as Tc sensitive, those growing on 5 µg ml⁻¹ but no higher, as non-recombinant plasmid vehicles, and those forming colonies on higher than 5 µg per ml Tc were categorised as recombinants exhibiting promoter activity. In cases where more than one frequency is indicated, the experiment was repeated and both results are listed. In every instance the remainder of the transformation mixture was plated onto plates containing 10 µg per ml Tc and 20 µg per ml Ap in order to select only those recombinants demonstrating Tc resistance greater than the plasmid vehicle. By spreading an aliquot of the cells on Ap plates alone at the same time, we could determine the proportion of transformants represented by these promoter-active recombinants. Based on the data obtained from patching the Ap-resistant transformants, we calculated the percentage of total recombinants which showed promoter activity. The results obtained were comparable with those listed in table 1. Since each experiment was carried out in identical conditions with respect to amounts of DNA used and time of incubation after transformation, it was not necessary to adjust the data for such factors as selective advantage, sibling production and genome size.

of pBRH3B. Ampicillin resistant transformants of *E. coli* were selected and then examined for increased levels of Tc resistance. We collected a series of recombinant plasmids for each type of DNA cloned which expressed Tc resistance levels ranging from complete Tc sensitivity, to resistance greater than 5 µg ml⁻¹ Tc.

For each experiment, the percentage of recombinants which exhibited Tc resistance was calculated (Table 1), and DNA from 5% of the recombinants was purified so that the presence of a foreign DNA fragment in pBRH3B could be verified by *Eco*RI digestion and agarose gel electrophoresis. A wide range of fragment sizes was observed, but no correlation between the Tc resistance levels and the sizes of the DNA fragments were found. It is evident from the data in Table 1 that the frequency of cloned promoter active fragments in pBRH3B is reproducible and depends on the origin of the DNA. Moreover, the data suggest that both yeast and *Euglena* chloroplast DNAs may harbour nucleotide sequences similar to the promoter regions present in prokaryotic DNAs. The low frequency of promoter fragments cloned from *Neurospora* and *Drosophila* DNAs stands in marked contrast to the yeast and chloroplast frequencies, and suggests that the nucleotide sequences of *Neurospora* and *Drosophila* may have evolved further from the prokaryotes than those of *Saccharomyces* or *Euglena* chloroplast DNA. Since most promoter regions sequenced thus far are usually (A+T)-rich¹, the low frequency of promoter fragments obtained from *Rhizobium* DNA in comparison with those of the other prokaryotic DNAs may be explained by the unusually high (G+C) content (64%) of *Rhizobium japonicum* 110 (ref. 4).

It is not yet certain that the regions on the eukaryotic fragments which are recognized by *E. coli* RNA polymerase are

bona fide eukaryotic promoters. Nevertheless, preliminary indications that *E. coli* RNA polymerase can recognise eukaryotic promoter sequences come from studies involving the cloning of the *Eco*RI fragment carrying the yeast 5S rRNA gene⁵ into the *Eco*RI site in pBRH3B. This fragment exhibits strong promoter activity, as manifested by the high level of Tc resistance conferred by the recombinant. When the orientation of the fragment in the plasmid is reversed, resistance to Tc disappears (unpublished observation). This observation could be explained if one presumes that one end of the *Eco*RI 5S fragment carries a promoter which can effect transcription of the Tc resistance gene. The notion that eukaryotic DNA sequences can serve as promoters in *E. coli* is further supported by studies in which functional expression of eukaryotic genes in *E. coli* was obtained. The *Neurospora crassa* gene, 5-dehydroquinase hydrolase (qa-2⁺), when cloned in the plasmid pBR322, can complement the *E. coli* mutation, *aroD6* (ref. 6). Moreover, similar results have been obtained with the yeast gene *his3*, which has also been cloned by complementation in *E. coli*⁷. The fact that expression of the *Neurospora* (S. R. Kushner, personal communication) and yeast⁷ genes in *E. coli* is independent of the orientation of the restriction fragment, suggests that transcription is being initiated by *E. coli* RNA polymerase at some point on the eukaryotic DNA and not by a plasmid promoter.

Previous efforts to identify eukaryotic genetic control signals by probing native or reconstituted chromatin with *E. coli* RNA polymerase have yielded mostly equivocal results. The experiments described here, however, offer an *in vivo* alternative to traditional *in vitro* modes of examining eukaryotic DNA. The fact that this approach does not require the functional expression of cloned genes makes it more desirable than cloning eukaryotic genes by the complementation of *E. coli* mutations. We feel that this kind of analysis will be useful for the isolation and characterization of promoters from organisms other than *E. coli*. The strength and number of promoter active fragments cloned can be affected by many factors, so it is possible that: (1) Tc resistance levels do not accurately reflect promoter efficiency and (2) due to sibling production, the percentage of promoter active fragments shown in Table 1 may only approximate the real percentage of *Eco*RI fragments carrying promoter active sequences. It is the relative frequencies of promoter active fragments which make this system amenable to the study of the evolution of regulatory sequences from prokaryotes to eukaryotes. Indeed, the frequencies of promoter active fragments obtained from *Saccharomyces* and *E. coli* DNAs suggest a degree of relatedness greater than might be expected for two protists separated by the prokaryote-eukaryote phylogenetic barrier. To try to gain further support for this analytical approach, we are extending this survey to include DNAs from additional prokaryotic and eukaryotic organisms.

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matters arising

Viking Mars labelled release results

THE labelled release (LR) Mars experiment^{1,2} yielded a positive response from Mars soil when a radioactive organic solution was added and a negative response when the soil was heated to sterilisation temperature. After storage of the soil at 10 °C for two to three months in the spacecraft, there was almost no response on addition of radioactive nutrient³.

Nussinov *et al.*⁴ proposed that LR Mars life-detection response arose from water-induced outgassing of CO₂ from Mars surface fines. They state that the kinetics of outgassing in LR and GEX are similar and that the characteristic time of the yield of O₂ in GEX and CO₂ in LR are also similar.

We are surprised that they ignored the fact that the CO₂ released by the LR is radioactive and, therefore, must have arisen from the radioactive nutrient added to the soil sample. Further, we do not agree that the reaction kinetics in GEX and LR are similar. GEX outgassed all of the measured O₂ in ~2 h whereas the half-time for the LR production of radioactive CO₂ was ~8 h, after which production tapered off, but continued slowly, for the duration of the particular experiment (up to 90 Sol).

Although we are not yet certain whether the LR response was biological or chemical, we are sure that it cannot be explained by the outgassing of CO₂ trapped in Mars surface fines.

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NUSSINOV *ET AL.* REPLY—We should like to correct here a false impression that our paper¹ contained a conclusion about registration in the LR experiment of 'CO₂ trapped in Mars surface fines'. We assumed that due to the radioactivity of the ¹⁴CO₂, such a conclusion could never be drawn either by us or by readers. No conclusion as such can be made about the origination mechanism of the registered gases on the basis of the GEX and LR kinetics. It is only natural to think that

¹⁴CO₂ resulted from interaction between nutrient and O₂, the latter developing from the soil's reaction with water. The time trend of the count curve is typically filtrational which means that the formation of O₂ was rapid as compared with its transport. From a classical physical viewpoint, their kinetics implies quantitative similarity only of GEX and LR curve shapes, itself implying identity of the power dependence ($\sim t^{1/4}$ at small t) and exponential saturation ($t \rightarrow \infty$). Qualitative differences are easily explained by the fact that the very designs of GEX and LR were incorrect from the physical standpoint, namely: (1) shapes (and masses) of the GEX and LR samples were not identical; (2) specific quantity of nutrient differed in the experiments; (3) the most informative initial segments of the kinetic curves were not registered. These are the reasons that it was impossible to expect a better than order of magnitude agreement. Therefore the similarity of the GEX and LR kinetics should undoubtedly be considered as fact.

Note that the data by Levin and Straat on 'almost no response upon addition of radioactive nutrient' after storage of the soil at 10 °C for two to three months, are readily explained by our model. Indeed, O₂ physically adsorbed within the micropores at elevated temperatures can be converted to a chemisorbed state, thereby losing its reactivity. At low Martian temperatures the chemisorption of O₂ is inhibited¹.

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Mechanisms of sputtering

THE letter by Pollitt *et al.*¹ on sputtering makes an interesting suggestion but also several incorrect assertions about the adequacy of existing theories and ignores other publications.

In currently accepted theories of the sputtering of metals the incident ion generates collision cascades amongst the target atoms^{2,3}. If these intersect the surface, atoms receiving more than a binding energy E_b , related to the heat of sublimation, may be ejected and appear with a

characteristic energy distribution having a peak at $E_b/2$ and falling off as E^{-2} at high energies of ejection E . Integration of the distribution leads to the total sputtering yield which is inversely proportional to E_b and directly proportional to the energy deposited by the incident ion in generating atomic recoils near the surface. In a crystal the lattice imposes a structure on the spread of momentum in the cascade, focuses momentum into close packed directions and leads to anisotropic emission.

The cascade lasts for a very short time, of the order 10^{-13} s. Afterwards it leaves a local heating effect in a region known as the thermal spike which may persist for up to 10^{-11} s. In cases where the density of energy is high enough and the binding energy is low enough one expects sputtering by evaporation into a characteristic energy distribution, something like a maxwellian with a peak at a few tenths of an eV^{4,5}. The spike temperature and hence the sputtering yield depends on the ambient temperature of the solid⁶.

These two basic mechanisms are complementary to one another, not alternatives as Pollitt *et al.* seem to suggest. The cascade component usually dominates unless the solid is at a temperature approaching its melting point, the heat of sublimation is low or the bombarding ion is of such a mass and energy as to produce cascades of high energy density. The main observations are in accord with these predictions contrary to the assertions of Pollitt *et al.*¹. The energy spectrum of sputtered atoms closely follows E^{-2} at high energies over many decades, generally has a peak at half the sublimation energy^{2,7-11} and develops a low-energy peak near 0.1 eV when thermal spike conditions are fulfilled^{2,12,13}. The total yield for various elemental targets with a fixed ion and energy is inversely proportional to sublimation energy. (The dependence on ion species for fixed target is a much more complex problem involving ion implantation phenomena)¹⁴. From single crystal targets anisotropic emission is observed¹⁵⁻¹⁷ with angular widths¹⁸⁻²⁰ and energy distributions^{2,21} consistent with momentum focusing effects. The measurements of yield over several decades of ion energy, when thermal spike conditions are not fulfilled, accurately follow cascade theory³. They definitely do not correlate with the energy deposited in electron excitation, which they should if the model proposed by Pollitt *et al.*¹ were operating.

In insulators, and particularly alkali

halide crystals, the characteristics of cascade and thermal spike mechanisms have also been seen¹³. But there is an additional mechanism at work which is related to electron excitation, because sputtering can be produced by low energy electrons and photons²². This has satisfactorily been explained^{23,24} in terms of an exciton model in which a Cl_2^- ion is produced with anti-bonding orbitals which cause the sputtering, in common with the model of Pollitt *et al.*¹. Unlike their idea it has been worked out in some detail and I am surprised they do not refer to it.

In the sputtering of adsorbates, molecular solids and polymers, experimental data are scattered. The fact that large molecular fragments are sputtered and the expectation that electronic relaxation times are relatively longer than in metals gives the model by Pollitt *et al.*¹ a better chance. An obvious test would be to search for correlation between sputtering yield and the electronic stopping power for the incident ion, perhaps by varying the ion energy over several decades straddling the energy where electronic stopping power is a maximum (say 10 keV–1 MeV H^+).

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Tectonic emplacement of ophiolitic rocks in the Precambrian Mona Complex of Anglesey

THORPE¹ believes that the Anglesey serpentinite–gabbro suite should be regarded as part of an ophiolite—and therefore tectonically emplaced—because of alleged similarities with Tethyan

ophiolites. The latter embrace a large and diverse collection of phenomena, so that it is hardly surprising that some parallels can be drawn. However, other features seem to have been misrepresented by Thorpe and critical differences neglected.

Apart from occurring on a scale quite minute in comparison with Tethyan and other ophiolites, the Anglesey suite (exposed not at Newborough, but near Rhoscolyn, grid ref. 277770) does not display the diagnostic properties of an ophiolite. For example, the serpentinite and gabbro show no semblance of a layered or sequential arrangement, there is no sheeted dyke complex, and although cherty sediments and pillowed basalts do appear on Anglesey they are distant, both geographically and stratigraphically, from the intrusive suite.

Thorpe asserts that aspects of the Anglesey suite such as ‘finely preserved igneous textures’ near margins, and ‘absence of evidence for regional thrusting’ are of ‘widespread occurrence’ in many tectonically-emplaced ophiolite complexes. It seems to me that many ophiolites (see ref. 2) are characterised by a peripheral tectonite fabric, at least at the basal margin, and that geological maps of ophiolite terrains are replete with thrusts.

I disagree that the suite ‘provides evidence of local rapid uplift, erosion and transport’. The country rocks are, on all sides, the same, distinctive, deep-water lithology which constitutes much of West Anglesey. Debris from the suite is not found (unlike Tethyan instances). The melange (olistostrome) occurring elsewhere on Anglesey evinces such processes arising later, but it also seems to contain no fragments of the serpentinite–gabbro suite, which may have still been at depth.

I have agreed³ that the overall Monian association suggests an ultimate oceanic or upper mantle origin for the suite, but unfortunately the geochemistry cited by Thorpe reveals nothing about how the material was emplaced. A comparison with other, such as Tethyan, ophiolites would imply emplacement by obduction, although Thorpe seems to envisage some (unspecified) mechanism associated with plate subduction. The field evidence is most compatible with the suite being a deformed magmatic peridotite–gabbro complex.

Applying the fashionable soubriquet ‘ophiolite’ to the Anglesey suite requires a worrying stretching of the definition, and introduces serious implications for the stratigraphy and tectonics of the Mona Complex as a whole.

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Extending the range of radiocarbon dating

THE letter from Hedges and Moore¹ on laser enrichment of ^{14}C and its application to radiocarbon dating describes a most interesting and valuable line of research deserving the acclaim of all concerned with late Quaternary studies. It may be worth pointing out, however, that their statement of their expectation that the laser enrichment and new mass spectrometric methods will allow radiocarbon dating to be applied “well into the last interglacial period” begs a chronometric question for the dating of the last interglacial is not yet known with certainty. A more cautious and less pre-emptive statement might be to the effect that the new methods, by extending the far limit of radiocarbon dating to 100,000 years, may allow at least a part of the last interglacial to be absolutely dated. This is a different matter and in view of the importance of Hedges’ and Moore’s work the distinction is worth making clear at the outset.

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HEDGES AND MOORE REPLY—The distinction made by Burleigh is well taken. In this context it should be pointed out that the absolute dating to which he refers can only be achieved after reliable calibration of the radiocarbon time scale for this period. Fortunately there are grounds for believing that corrections to the radiocarbon time scale will not be very large¹.

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Failure to produce hepatic hyperplastic nodules in rats by portacaval anastomosis and testosterone

WEINBREN AND WASHINGTON¹ recently reported the spontaneous development of hyperplastic nodules in the livers of rats previously subjected to portacaval anastomosis (PCA). The nodules were found in all rats by 6 months post-operation. Others have failed to note such nodules in PCA rats and the authors suggested that this may be related to the difficulty in detecting the lesions microscopically or to differences in rat strains, housing or diet. We have examined 24 PCA rats 10–15½ months post-operation and have not found liver nodules. Because

Table 1 Effects of portacaval anastomosis and testosterone in rats: autopsy data

	n	Body weight (g)		Mean change in body weight (%)	Relative organ weight (% of body weight)			Pale cells	Zone III atrophy	Bladder calculi
		Initial	Autopsy		Liver	Kidney	Spleen			
Control	9	637	659	+3.6	3.46	0.68	0.25	0	0	0
PCA (P)	9	492	475*	-2.0	2.26*	0.78	0.31‡	4	3	3
Testosterone (T)	7	661	585*	-8.4	3.77	0.86†	0.22	0	0	0
PCA + testosterone (PT)	8	486	429*	-11.6*	2.65‡	1.3	0.36‡	4	4	5
			†(PT-T)	†(PT-P)	(PT-P)	†(PT-P)				

* $P < 0.001$; † $P < 0.01$; ‡ $P < 0.05$.

Weight changes are those from beginning of testosterone treatment. P values are for comparison of treatment against control unless specified (PT-T, PT-P), using two-tailed t -test.

it is known that some androgens produce adenomas in humans² and facilitate the development of hyperplastic nodules in carcinogen-fed rats³, we also tested the effect of testosterone (T) on PCA rats.

Male Wistar rats (Charles River) approximately 250 g were subjected to PCA or sham operation over a 2½-month period. Others were kept as controls. All were maintained on Purina rat chow Formulab and tap water *ad libitum* in wire bottom cages, four or five per cage. Five PCA rats killed 10 months after operation did not have nodules. Half of the remaining rats were given 200 mg testosterone enanthate (Delatestryl; Squibb) in sesame oil subcutaneously at monthly intervals for three doses. The other half received oil only (Table 1). There were six intercurrent deaths (two PCA, two PCA+T, two T); three of these animals were autopsied and found to be free of liver nodules. The remaining animals were killed one month after the last dose and 13½–15½ months (mean 14½) after operation. Under ether anaesthesia, Evan's blue in saline was injected into the spleen and the anastomosis was considered satisfactory if the liver did not darken within 10 s. Otherwise the animals were rejected from the study. Sham and control animals showed no significant difference and the data were pooled.

The livers were examined carefully for gross or microscopic nodules. None were found in any group. PCA decreased the body and liver weights and increased the spleen weights whether expressed as absolute or relative weight (% of body weight). Atrophy of zone III hepatocytes with an increased nucleo-cytoplasmic ratio in these cells compared to zone I cells was seen in 7 of 17 PCA±T rats. Scattered hepatocytes with larger vesicular nuclei and pale cytoplasm lacking the usual coarsely granular basophilia were seen in 8 of 17 PCA±T rats (Table 1). These cells were located in plates one cell thick and were not clustered to form nodules. The spleen in the PCA±T groups had follicles similar in size and character to those of controls but there were more lymphocytes and occasional plasma cells in the cords of the PCA spleens. Bladder calculi, often accompanied by pyelonephritis, were common after PCA.

Testosterone caused yellowing of the fur, gross enlargement of the seminal vesicles and a decrease in the body weights. There was a relative increase in liver weight in the PCA group and absolute as well as relative increase in kidney weight in both PCA and control groups.

PCA in this study induced liver atrophy but not hyperplastic nodules. Strain and dietary differences are likely explanations for the discrepancies between this work and that of Weinbren and Washington.

We thank Dr Fred Plum for the animals.

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On codon usage

THE total nucleotide sequence of the *lac* repressor gene *I* (361 codons assigned) has recently been determined by Farabaugh¹. He has found that the codon usage is quite different from that of bacteriophage MS2 and Φ X174 (1,068 and 1,346 codons assigned, respectively). Nevertheless, assuming that both the repressor protein and the phage proteins are synthesised rapidly, he concluded that the codon usage presumably does not reflect a difference in efficiency of translation. As mentioned also by Farabaugh, however, no firm data actually exist on the relative translational efficiency. Moreover, even a small effect, difficult to reveal by direct methods, can have a strong selective value when judged over many generations.

Bacteriophage MS2 produces a burst size of 5,000 to 10,000 plaque-forming units (PFU) per cell, and each virus particle contains 180 coat protein molecules. Consequently, we have argued before that MS2 has optimised translational efficiency, especially of the coat cistron: one of these ways, among others, is by the proper choice of degenerate code words^{2,3}. It is likely that there exists an optimal interaction energy between the

codon and the cognate anticodon. As a result, codons of the type (A/U)–(A/U)–Y—where the first and second letters give an intrinsically weaker interaction—would be stabilised if the third letter is C. Indeed, in MS2RNA the N-N-C codon is preferred over N-N-U for all codons of this type (Phe, Ile, Tyr and Asn)⁴. Conversely, intrinsically strongly interacting codons of the type (G/C)–(G/C)–Y should be loosened by a preference for U. In fact, N-N-U codons are preferred over N-N-C for Pro, Ala and Gly (Arg codons are not included as they are recognised by an inosine-containing tRNA).

When we now consider the codon usage in the *lacI* gene, which makes the *lac* repressor protein at very low levels, we find exactly the opposite situation. U as a third letter is preferable to C for codons of the weak interaction type (Phe, Ile, Tyr and Asn); this is remarkable, considering that of third letters 28.6% are C and only 21.1% are U. Conversely, for the codons with a strong interaction in positions 1 and 2, the third letter is preferably a C rather than a U (Pro, Ala, Gly). This selective pattern of codon usage in the two systems is very striking and it would be of considerable interest if it could be related to a difference in translational efficiency. Calos⁵ has shown that the low level of *lacI* expression can be explained by a low-efficiency promoter; as pointed out by Müller-Hill *et al.*⁶, one can also easily imagine that the low level of constitutive *I* expression is further reduced by some limiting step in the translation processes, such as selective codon usage.

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reviews

Nature/nurture between the Wars

William J. Schull

The Triumph of Evolution: American Scientists and the Heredity/Environment Controversy, 1900–1941. By Hamilton Cravens. (University of Pennsylvania Press: Philadelphia, 1978.) \$17.50.

HAMILTON CRAVENS' *The Triumph of Evolution* describes the sequence of events in the United States between the two World Wars which he contends led to the resolution of the heredity-environment controversy. Whether, indeed, a resolution has occurred, as he asserts, may seem dubious to one who follows *Nature's* book pages and the controversy over the roles of nature and nurture in differences in intelligence, or whatever it is that intelligence tests measure. Resolution here, however, merely means the achievement of an agreement among natural and social scientists that nature and nurture are distinct but interdependent variables.

The central theme of this book is that the sudden increase in the last decade of the nineteenth century of experimental biology and psychology, aided by the emergence of the new graduate universities, fostered an academic professionalisation of these sciences which had and continues to have profound repercussions. It led to changes in educational emphases in these institutions and encouraged the development of the accoutrements of science which we now take for granted. Numerous professional societies and journals were born, and a scurry for research resources begun which persists unabated. Out of this process, Cravens maintains, came consensus. A little lamented casualty was social Darwinism, at least in its most strident forms, whose adherents waned as the new professionals grew in number.

A complicated, somewhat conjectural but nonetheless intriguing series of events and developments in anthropology, genetics, psychology and sociology is set forth to support this central thesis. Much of Cravens' argument emerges through an analysis of the actions and inactions of the prominent figures of the time in the aforementioned disciplines. Some of these individuals intrigue him more than others. Franz Boas is such a case, but Davenport, Morgan, Ross, Yerkes,

Dewey, Watson, Thorndike and Cattell—to mention but a few others—have their moments of attention. The activities of these individuals and their students are often interpreted in terms of their ethnic, religious and social affiliations. Their contributions to their branches of science as well as their academic and social fates are briefly limned. Most are treated sympathetically with possibly Charles Davenport emerging as the most prejudiced, least principled and least qualified of the major personalities. Familiar lesser figures such as Madison Grant, Harry Laughlin and Henry Goddard receive short shrift as is their due. To these three are ascribed some of the more sordid chapters in the eugenics movement in the United States and rightly so.

Cravens' is not the first analysis of many of these events. Kenneth Ludmerer's *Genetics and American Society: A Historical Appraisal* (1972) and Mark Haller's *Eugenics: Hereditarian Attitudes in America Thought* (1963), for examples, trace many of the same developments, and often somewhat more thoroughly but from quite a different perspective. This book makes more of the events which were occurring in the new universities, their strivings for distinction among their peers, and the emergence of a scientific community in the New World not wholly dependent upon that in the Old for its directions and philosophy. It prompts thought on the unusual impact that a few forceful personalities may have upon a discipline; witness Boas' imprint on anthropology.

Cravens' analysis does not strike me as especially disputatious. It is thoughtful and built on a serious consideration of a variety of materials—biographies, letters, journals, society proceedings and the like. Few will be able to read *The Triumph of Evolution* and not be exposed to new facts or facets in this controversy. The geneticist will learn of the turmoil his science provoked in the social sciences, and should be able to appreciate positions which may have merely seemed obdurate in the past. Similarly the cultural anthropologist, say, should learn more of the history of twentieth-century genetics, and the origins of its collective perspective. All will be exposed to parallelisms of which

most readers will have been previously unaware. Many will be tempted to read further, and the extensive notes with which the book concludes will prove invaluable.

This otherwise interesting and generally unexceptionable book is marred in my view by two shortcomings. First, it is unnecessarily repetitive. Much of the contents of the chapter "The New Biology" is repeated elsewhere. We are told, for example, on p24 of G. Stanley Hall's strengths and weaknesses as the President of Clark University and much the same material appears again on p65. Edward East receives similar double treatment on pp51 and 169. Others fare in like manner. There is moreover a tiresome repetition of labels which brings me to my second objection. *The Triumph of Evolution* is flawed by the frequent use of stereotypes of people and institutions. We read of the white, Anglo-Saxon, Protestant; the left-liberal; the gentle Quaker and the humble normal school. While these may be handy labels and often serve a useful, albeit pejorative purpose in political tracts, they seem out of place to me in an historical account of this nature. H. J. Muller, for example, is variously characterised as brilliant, a radical, a man with left-liberal tendencies. Most who knew him would accept all of these appellations as valid. But he could equally well have been described as an elitist, an uncommonly gifted investigator with an incredible biological intuition, or a teacher who could communicate the excitement he found in genetics to students and colleagues alike. But do any of these labels provide a compelling basis for characterising him as one who was "quite willing to tolerate the eugenics movement and to perhaps even support some of its goals so long as the movement appeared disinterested, scientifically credible, and dignified"? I'm not questioning the attribution but merely its justification on no more forceful evidence, seemingly than a series of labels. These are poor substitutes for a penetrating analysis of the man. □

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Microtubules

Microtubules. By P. Dustin. Pp. 452.
(Springer: Berlin, Heidelberg and New York, 1978.) DM148; \$74.

It is now fifteen years since Slaughterback first coined the term 'microtubule', and the literature has grown expodentially from a mere two papers in 1963 to several hundred per annum today. During this time, there have been intermittent review chapters and specialised monographs originating from symposia, but never a survey encompassing the whole field. Finally, Dustin has written a book which deals exclusively with microtubules, which should be in every library, and which will be obligatory reading for everyone interested in microtubules.

The first half of the book is primarily concerned with the structure and biochemistry of microtubules, the interactions between microtubules, and microtubule inhibitors, whereas the second half concentrates on aspects of cellular physiology in which microtubules have been implicated, such as movement, secretion, axonal transport, and of course, mitosis. The book is not without its faults: it is for example exclusively a summary of the extensive literature and is not a critical appraisal of the work.

Furthermore, it has taken two years to produce since Dustin completed his search of the literature, and so inevitably many current ideas are absent. One example is that the book only briefly mentions microfilaments and lacks any discussion of a possible interaction between microfilaments and microtubules. As such it is interesting to see where we were at two years ago, in the same way that the author's earlier book (with Eigsti), *Colchicine*, is a fascinating account of the field a decade before the visualisation of microtubules. A more serious fault is the review of the literature concerning the biochemistry of microtubules and the kinetics of assembly/disassembly *in vitro*, which is superficial and which fails to relate the properties *in vitro* to the *in vivo* behaviour of microtubules, yet clearly our understanding of the role of microtubules in cellular events will develop from such comparative studies.

Finally, the book is written as a series of independent chapters, each with its own bibliography, with the consequence that it is difficult to search for specific references. Despite these reservations, this is a book that should be read by all cell biologists. **Roy Burns**

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Autoimmunity

Autoimmunity: Genetic, Immunological, Virologic and Clinical Aspects. Edited by N. Talal. Pp. 734.
(Academic: New York, San Francisco and London, 1977.) \$48.

AUTOIMMUNITY was considered thirty years ago as a contradiction in terms. If immunity, then regarded almost entirely to be due to antibodies, eliminated or neutralised microbes and other foreign substances which had penetrated the body it would be terribly dangerous to make antibodies against one's own tissues. Furthermore, no-one had convincingly shown that antibodies against self components were normally ever made. Antibodies could indeed be induced which reacted with an animal's own spermatozoa, lens protein or even brain tissues, but it was argued (sometimes correctly) that these were exceptions, as the antigens involved were in fact excluded from contact with cells of the immune system; and when brought into contact by infection or by injury they were essentially foreign. With improved techniques, however, undoubted autoantibodies were described in an increasing number of human diseases, such as haemolytic anaemia, thyroiditis, pernicious anaemia

and rheumatoid arthritis. Autoantibodies became respectable, although it was still uncertain in most cases whether they caused the disease or were a secondary consequence of tissue damage, which had resulted in liberation of specialised tissue components that were normally hidden intracellularly but were recognised once they entered the blood stream.

The notion that autoimmunity did not occur—expressed by Ehrlich's term 'horror autotoxicus'—had been so widely accepted that few had bothered to question how the body could distinguish self from not self. In terms of current biochemical knowledge the phenomenon was inexplicable, but the clonal selection theory put forward by Burnet in 1959 made sense in terms of cell populations. It proposed that all lymphocytes potentially able to make antibodies against self antigens were eliminated if they met them during foetal life, and that auto-antibodies were the products of 'forbidden' clones, which had undergone somatic mutation after the period of obligatory development of self tolerance was over. This explanation had the added advantage of accounting for the increasing incidence of autoimmunity with age. However, in immunology few hypotheses remain intact for long. It was

found that normal animals could quite easily be induced to develop auto-antibodies against thyroglobulin by immunising with cross-reacting thyroglobulin, that inbred strains developed anti-thyroid antibodies spontaneously, and that normal animals contained lymphocytes with surface immunoglobulin receptors specific for thyroglobulin (and for other self components) even though they made no detectable antibodies. This implied that a considerable number of forbidden clones could not have been eliminated.

New light was shed when it was realised that specific T lymphocytes, recognising the 'carrier' part of antigens, are required to cooperate with B lymphocytes for the latter to secrete antibodies against a particular antigenic determinant. T lymphocytes are more easily inactivated by contact with antigen; the specific helper T lymphocytes having been eliminated, the B lymphocytes able to make autoantibody would only do so if an alternative set of helper cells were available. These could be provided by stimulating with cross-reacting antigens or self antigens altered in some way, such as by viral infection. Absence of autoimmunity could now be attributed to lack of helper T cells. Next came the recognition of the existence of suppressor T

cells, which could prevent helper cells from being stimulated or from functioning. Certain strains of animal unusually prone to autoantibody formation were found to show premature loss of suppressor cell function, and the regulatory role has been shifted to suppressor cells. Instead of self-reactive cells being absent we now have to consider them as normally present, but kept in check by an elaborate feedback system. Jerne's network hypothesis, whereby any lymphocyte specifically responding to an antigen may stimulate a specific response against itself, and so on, provides a theoretical model whose usefulness is being increasingly recognised and validated.

Against this background an up-to-date book on autoimmunity was needed. The contributors to the present multi-author volume are all experts. The topics covered in 23 chapters range from genetic control of autoimmunity in man and other species (including an interesting account of the history of NZB and NZW mice); ways in which autoimmunity can come about covering examples illustrating all the various mechanisms discussed in the introductory part of this review; role of suppressor cells; relevance of the

network theory; other feedback mechanisms, including blocking factors; correlation of autoimmunity with malignancy, immunodeficiency and with a variety of viral infections; special aspects of autoimmunity, including SLE, rheumatoid arthritis, diseases of the central nervous system, and antibodies against physiologically important receptors in thyrotoxicosis and myasthenia gravis; a very full account of the pathological effects of immune complexes; and a final chapter on how autoimmune (anti-idiotypic) responses can be used for positive and useful regulation. The level is advanced, but the language is reasonably clear and the book should be useful for clinicians as well as professional immunologists. The coverage is not complete (for example, there is little about haemolytic syndromes, skin diseases or the gut) but the aspects covered are done well. Bibliographies are more than adequate, and there is a reasonably good index. This book is the best and most up-to-date currently available.

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Nuclear power engineering

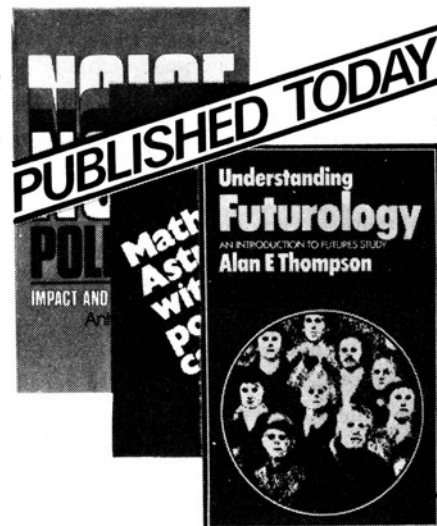
Nuclear Power. Vol. 1: Nuclear Power Plant Design. Vol. 2: Nuclear Power Project Management. By Erik S. Pedersen. (Ann Arbor Science: Ann Arbor, Michigan; Wiley: Chichester, UK, 1978.) £18.60 each volume.

In recent years nuclear engineers have undoubtedly suffered from a surfeit of excitement. After the oil shock of 1973 nuclear engineers were acclaimed as potential saviours, and almost simultaneously accused of plotting the obliteration of the planet. Nuclear engineers have seen their industry expand at breathtaking speed, only to watch now as it threatens to subside like a punctured balloon. Nuclear engineers hear politicians proclaiming the wonders and prestige of nuclear enterprise, while ducking as the groundlings throw eggs. Nuclear engineers might well welcome a little boredom. If so, may I prescribe two volumes of Erik Pedersen, taken at bedtime?

Given a topic as potentially engrossing as nuclear energy—fascinating phenomena, virtuoso engineering, daunting challenges of every kind to be met—it cannot have been easy to write 950 pages of unrelenting tedium. But both volumes of Pedersen's *Nuclear*

Power read like computer printouts, an avalanche of factual information recited in a dogged monotone, in which even the most ingenious intricacy is embalmed lifeless on the page. Indeed both volumes of *Nuclear Power* read disconcertingly like each other. The first is called *Nuclear Power Plant Design*, the second *Nuclear Power Project Management*; but perhaps one-quarter of the text in the second volume is lifted verbatim from the first, without any apparent indication of this repetition. The unwary purchaser may react more emotionally to the text when he realises belatedly that he has unwittingly paid for two helpings of it.

It is not, in any case, clear just who is expected to read the books. The Preface to each volume opens with the statement that it "is intended to be used as a working reference book for management, engineers and designers, and as a graduate-level text for engineering students. The book is designed to combine theory with practical nuclear power engineering and design experience, and to give the reader an up-to-date view of the status of nuclear power and a basic understanding of how nuclear power plants function". A much better writer than Mr Pedersen would be hard pressed to find a level at which all these disparate objectives could be even minimally met. As a result both volumes of *Nuclear Power* include far too much material that



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would go far over the head of the reader desiring "a basic understanding"—unexplained references to "eigen-values" and other jargon, and some of the most hair-raising equations *ex machina* ever put to paper, with no hint of derivation or underlying assumptions—while omitting to provide essential detail which any responsible designer, engineer or manager would require before making use of much of the information in the books.

Volume 1 opens with a chapter on nuclear reactor theory, which at once undermines the reader's confidence by getting a well-known number wrong: the Trinity test took place not on 6 July but on 16 July 1945. It may be merely a typographical error, but any book with as much undefended numerical data as this cannot afford such a demoralising error on page 2. This and subsequent chapters might serve as a recapitulation for those who already know the subject and simply need to have their memory refreshed; but the explanations will require much expansion by any student using the book as a first-time text. The questions at the chapter-ends might have been drawn from the author's draft outline: "1. Explain the buildup of atomic structure. 2. Explain the atomic number and mass number. 3. What is an isotope? . . ." and so on. A student could regurgitate the foregoing text verbatim—but might not have the faintest idea of its meaning. The chapter on 'Nuclear reactor design' uses the word 'design' as a noun, not as a verb; nowhere is there any effort to discuss the actual process of nuclear design. Instead there are reams of description, naming of the parts. There are chapters on 'Types of nuclear power plants' (mostly repeated in Volume 2) and licensing (ditto, and entirely taken up with US procedures), on shielding, containment, steam and turbine cycles, electricals, instrumentation, and radioactive waste management. Volume 2 also includes chapters on safety analysis, project services, quality assurance, scheduling and plant operation, nuclear fuel management and plant cost management. Appendices list terminology and US regulatory guides. Unfortunately the index is skimpy, making the books less useful even as a concise nuclear encyclopaedia.

The economic information is alarmingly erratic. "The annual fuel expense equals about \$25/kWe, which, over 30 years of plant life, implies expenditures of \$750/kWe for nuclear fuel": only if you buy all the fuel at the beginning, or ignore the entire process of discounting and the economic track record of the civil nuclear industry. Similar flat statements of purported economic fact could be disputed at great length. So could the declaration

of faith with which the author concludes the books: "Nuclear power is the safest, cleanest and least expensive energy source available today and in the foreseeable future. We must, therefore, take advantage of it." Given the losses so far sustained by those engaged in civil nuclear power worldwide, it is not clear who is taking advantage of

whom. However, it is unhappily clear that these two volumes will do little to raise the flagging esprit de corps of Mr Pedersen's colleagues, nor entice fresh blood into the enterprise.

Walt Patterson

Walt Patterson is energy consultant to Friends of the Earth (London) Ltd.

Annelid physiology

Physiology of Annelids. Edited by P. J. Mill. Pp. 683. (Academic: New York and London, 1978.) £28; \$58.

THIS book should probably be compared with the Annelid volume of Florkin and Scheer's series on Chemical Zoology, the closest thing we have had until now to a volume on annelid physiology. Happily, only 5 of the 14 chapters in Mill's volume cover topics previously reviewed, and only two of these are by the same authors (Clark on systematics and Oglesby on osmoregulation). Comparable reviews must otherwise be sought in volumes devoted to specific physiological systems (for example, respiration and reproduction); there, in fact, they may be somewhat more useful, as physiologists are more often interested primarily in their physiological specialty and less frequently interested in results in all fields of physiology from a specific group of animals.

The volume has unfortunate technical flaws (smeared print on a number of pages in my copy) and numerous distracting errors in grammar, punctuation, and figure labelling (especially in the editor's chapter) which detract from the flow of the text. These are in addition to frequent, obvious typographical errors.

Richards' chapter on the epidermis and cuticle is a welcome summary, although more might have been included on gland cell ultrastructure, and several erroneous statements on seta formation in reproductive forms of polychaetes are included. Mill provides a list of most of the information available on sense organs and their associated pathways, but offers little synthesis.

Tashiro and Kuriyama's mistitled chapter on neurosecretion is really a good review of synapses and neurotransmitters as they occur in annelids. Annelid neurosecretion in the neuroendocrine, peptidergic sense has been reviewed several times in recent years, but neurotransmitters have been somewhat neglected; thus, the chapter is more useful than the title would lead one to believe.

Olive and Clark's chapter on reproduction nicely complements the only other recent review of this subject. Division of annelid species into monotelic and polytelic groups (that is, those which breed only once and those which breed more than once) provides an interesting touchstone especially for the analysis of polychaete life cycles. It particularly illuminates the various possibilities for physiological control. Polychaete diversity is as evident in reproduction as it is in feeding habits and external structure and this classification offers an opportunity to analyse this diversity on a rational basis.

In separate chapters, Weber discusses both respiration and the respiratory pigments. His approach is functional and he provides a good summary of the abundant recent work, especially on the pigments and their function. The diversity of polychaete life styles and respiratory pigments provides interesting material for the investigation of physiological and biochemical adaptation, and enough work has now been done in this area to sketch the broad outline of different probable physiological strategies in different respiratory stress situations. Mangum provides a critical, constructive review of the related area of temperature adaptation. These chapters emphasise polychaete diversity and will doubtless command a good audience.

In an unusual chapter on defence mechanisms, Dales reviews several neglected, and somewhat disparate topics including wound-healing (a neglected component of regeneration), responses to both parasites and microorganisms, and, briefly, graft rejection and immunity.

Oglesby's exhaustive and critical review of salt and water balance physiology again emphasises polychaetes and their diversity. This chapter ought to stand for some time as the definitive statement of our knowledge in this area.

Considering the cost of a volume such as this, both the reader and the contributing authors have a right to expect better quality from both the editor and the publisher.

Paul C. Schroeder

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obituary

Manne Siegbahn, 1886–1978

MANNE SIEGBAHN, who died on 24 September 1978 at the age of nearly 92, was one of the great leaders in the development of experimental atomic physics during the first half of this century. He is known in particular for his pioneering work in X-ray spectroscopy, for which he was awarded the Nobel prize in 1925, and as an eminent organiser of research in experimental physics in Sweden, where he created and led very productive research groups at Lund (1914–1923), Uppsala (1924–1937) and Stockholm (1937–1964).

Siegbahn began his academic career in 1906 at the University of Lund where J. R. Rydberg was then professor in physics. His dissertation in 1911 (on magnetic field measurements) had no connection, however, with Rydberg's famous work on series in atomic spectra, nor had it any bearing on Siegbahn's future line of research. At that time, atomic physics was in a state of rapid development. In 1913, Bohr's model of the atom, based on Rutherford's nuclear atom and the radiation laws of Planck and Einstein, had quantitatively explained the hydrogen atom, but no clear path through the jungle of amassed data on other atomic spectra was in sight.

A more promising way of getting an insight into atomic structure seemed to appear when Moseley, in 1914, by using the newly discovered Bragg reflection from crystals was able to measure the wavelengths of the X-rays emitted by different elements and found their very regular dependence on the atomic number. Siegbahn saw the possibilities of this approach and immediately put the available resources of the laboratory in Lund into the development of X-ray spectroscopy. Showing for the first time his remarkable aptitude for instrument design Siegbahn developed to perfection the crystal spectrographs and the X-ray tubes as well as the vacuum technique which was a big experimental problem in those days. He transferred his own enthusiasm to his group of students, and the laboratory in Lund became a centre for X-ray spectroscopy which attracted researchers from many countries.

As a result of a decade of hectic activity the knowledge of the X-ray spectrum of different elements was en-



riched with many details, the range extended toward short and long wavelengths within the limits set by the crystals, a new series (M) was discovered and the wavelength measurements were improved step by step to a high degree of precision. For practical reasons the wavelengths had to be expressed in a special (Siegbahn) unit based on an assumed value for the lattice constant of the crystals. The experimental technique was described and the observational data collected in Siegbahn's book *Spektroskopie der Röntgenstrahlen* that went through several editions, each time revised and extended, and became the bible for workers in this field.

Having been acting professor and head of the laboratory since 1915 Siegbahn was appointed to the chair in 1920 after Rydberg's death. In 1923, however, Siegbahn accepted an invitation to the chair of physics at the University of Uppsala, where he soon assembled a new group of devoted students. Although X-ray spectroscopy remained for some time the main interest, other more or less related activities were initiated. Among them

was the determination of the fundamental atomic constants, leading, for example, to a considerable revision of Millikan's oil drop value for the atomic unit of electric charge.

Around this time it had been shown that X-rays could be diffracted by ruled gratings if the rays made a small enough angle with the reflecting surface (grazing incidence). Realising the possibility offered by this method to extend the observable range of X-ray spectra, Siegbahn built in 1928 a grazing-incidence concave-grating spectrograph which had the characteristic features of a Siegbahn design: it was simple, efficient and easy to work with. It proved its value and was followed by several similar instruments of increasing size. Besides amply fulfilling the hopes of extending the range of X-ray spectroscopy, these instruments effectively opened up the almost inexhaustible field of investigations into the 'optical' spectra of highly ionised atoms. The gratings used in these spectrographs were ruled on a ruling engine of Siegbahn's own design.

In 1937 Siegbahn moved to Stockholm to become director of a new research institute created for him by the Academy of Sciences with financial support from the Wallenberg foundation and the State. Established as a department of the Academy's Nobel institute it became generally known as the Nobel Institute for Physics.

Anticipating the coming importance of nuclear physics which began to develop during the first half of the 1930s, Siegbahn planned the new institute especially for research in nuclear physics which up to then had been almost completely neglected in Sweden. The institute was equipped with one of the first cyclotrons in Europe and with excellent facilities for nuclear spectroscopy. For the third time Siegbahn managed to assemble a large group of able collaborators and made the Nobel Institute a centre for nuclear research of high international reputation. Siegbahn formally retired from the directorship in 1964, at the age of 78, but continued to maintain a keen interest in the activities of the Institute.

Manne Siegbahn's success as a research leader was founded on a deep interest in the subject matter as well as a feeling for the potential fertility of a field and an unhesitating readiness

to take up new lines of research. As a designer of instruments he saw the essential functional requirements and found the most direct way of solving the problems. In his contacts with people Siegbahn was a man of few words, but what he said was always to the point and his quiet manner was not to be taken as a sign of timidity. On the contrary, he possessed the large amount of contagious self-confidence needed for bold enterprises. Siegbahn took little interest in formal teaching and he gave his research students a great deal of freedom in their work, at the same time keeping them aware of his generous encouragement.

Bengt Edlén

E. A. Moelwyn-Hughes

WITH the death, on 10 September 1978, of Emyr Alun Moelwyn-Hughes, Fellow of Darwin College and for many years Lecturer in the University of Cambridge, the world of chemistry has lost one of its luminaries.

He was born in Cardigan in 1905 one of the six children of the Rev. Dr J. G. Moelwyn-Hughes, a well known Welsh Presbyterian minister and hymnologist. After a remarkable career as a student in the University of Liverpool, who awarded him a DSc at the age of 28, and where his interest in kinetics was first aroused by Prof. W. C. McC. Lewis, he went to Magdalen College, Oxford as 1851 Exhibitioner.

It was there, alongside C. N. Hinshelwood with whom he collaborated, that his work on solution chemistry and the comparison of the rates of change in the gaseous and liquid state first flourished. His interests soon encompassed ionic reactions, enzyme catalysis, the isotope effect and the general theory of solutions. The first of his classical texts *The Kinetics of Reactions in Solutions*, which greatly influenced the subsequent development of the subject, was published in 1933, at about the time when he embarked on a year's collaboration with K. F. Bonhoeffer at the Institut für physikalische Chemie, Frankfurt.

In 1934 he moved to the University of Cambridge where, apart from the war years when he was associated with the Ministry of Supply, he remained for the rest of his life, except for short visits abroad. He held the Messel Fellowship of the Royal Society from 1936 to 1940, and later became a Vice President of the Chemical Society.

As a teacher in Cambridge, and as an extraordinarily successful author of several standard texts, he influenced the careers of countless young sci-

entists. One of his own research associates (J. C. Kendrew) became a Nobel laureate, and many others reached distinguished heights. They were taught at the time when his own experimental contribution was at its apogee: at a time when he wrote the often-quoted 'the complete physical chemist blows his own apparatus and solves his own equations.'

But he will be remembered best for his scholarly authorship of texts which were widely acclaimed. Moelwyn-Hughes wrote about his chemistry with the consummate skill of the accomplished creative artist. His dignified fluency, together with his wit, perception and perspective, was altogether refreshing in its originality and impact. Molecules, and other inanimate entities of the physical world, were frequently endowed with human qualities; and he contemplated the beauty, magic and mystery of their behaviour in the best traditions of the natural philosopher and poet. He had the innate feeling for the right turn of phrase. On listening to him, or reading his work, one was impressed by the freshness of his metaphors, which were invariably newly minted. And he obviously relished the act of regulating the rhythm of his sentences. His physical chemistry was described with the right words in the right order. Some of the gems that constitute part of his legacy trip off the tongue: 'Energy among molecules is like money among men; the rich are few, the poor numerous.' 'Belief in the essential simplicity of things is one of the chemist's articles of faith.' And consider his discussion of the self-evident fact that the liquid state of matter is intermediate between the solid and gaseous states. 'Like a central party in politics or a moderate denomination in religion, the liquid state is less rigorously defined and more difficult to understand than either of the extremes that flank it.'

His introduction to the Everyman Edition of *The Sceptical Chemist* (1963) is a model of succinct historical exposition. In asking when the discovery of the inverse relationship between pressure and volume was made and who was Robert Boyle, he wrote: 'He flourished in the seventeenth century, that turbulent time of pestilence and fire so amply described by Evelyn and Pepys, when Bunyan wrote in Bedford jail and Penn left England's shores, when Milton sang his *Paradise Lost* and Wren built London's churches, when Britain's monarch was overthrown and Cromwell made Protector.' His book-reviews were also delightfully composed.

Moelwyn-Hughes was greatly admired by many. He was appreciated as a significant figure in twentieth century

physical chemistry. His coruscating, if sometimes caustic, wit was much enjoyed (and occasionally feared) as was his superb storytelling gift; and very many including those Welshmen who got to know him at the University of Cambridge Welsh Society (Cymdeithas y Mabinogi), valued his warm-hearted friendship.

His illness was prolonged: for many years he was struck with immobility and incapacity. During that time he uttered not a syllable of complaint; and throughout it all he was nursed with profound affection and care by his devoted wife Mair who, along with his twin sons Edmwnd and Rolant, survive him.

J. M. Thomas

F. C. Fraser

DR FRANCIS CHARLES FRASER, CBE, FRS, Polar Medal 1942, a cetologist of international distinction and Keeper of the Department of Zoology at the British Museum (Natural History) from 1957 to 1964, died on 21 October 1978 at the age of 75. A graduate of Glasgow University, where his interests had been in both zoology and geology, his love of the sea and an appropriate opportunity sent him to the Antarctic and South Georgia from 1925-1933. His early work with the 'Discovery Investigations' was on the development and distribution of the young stages of *Euphausia* (*Discovery Rep.* 1936, 14, 1).

From the time of his appointment to the museum in 1933 as an Assistant Keeper, Fraser devoted his research to various aspects of cetology. He took over the *Reports on Cetacea, stranded on the British Coasts* which had been initiated in 1913 by Sir Sidney Harmer. This involved the development of communications between coastguards and other authorities and the museum, and then recovery, identification, measurement, and preparation of specimens to ensure the fullest scientific use of each stranded cetacean. Successive *Reports* were published in 1934, 1946, 1953 and 1974. Each contained analyses of strandings by species, site and distribution, number per month, estimated age, time of birth and summaries of many other findings useful in building up facts about the life histories of cetaceans. Altogether (from 1913-1966) 1,550 cetaceans were identified by Harmer and Fraser. These reports have been so valuable that other countries have begun to publish similar records.

During the Second World War, Fraser worked at the Admiralty. Afterwards he joined the 'Atlantide' expedition (1945-1946) and studied cetaceans off the coasts of West Africa. He soon confirmed his reputation as a leading

cetologist, especially on the often difficult decisions of identification: people from all parts of the world came for his help. His numerous taxonomical notes, mostly on rarer species, provided clarification where there was usually utter confusion.

In 1956 he described a species of dolphin *Lagenodelphis hosei* from a single skeleton collected before 1895 on a beach in Sarawak. From 1971 onwards sightings and captures have been made in widely scattered parts of the world to 're-discover' what is now known as Fraser's dolphin.

He contributed original observations on the anatomy, especially cranial osteology, and morphology of many cetacean species and made use of stranded animals brought to the museum both for research and display. The Whale Hall's present existence and its popularity owe much to Fraser's desire to educate the public about whales and dolphins. He also supported many of the early enterprises to exhibit live dolphins. His informative museum guides to British cetaceans and his splendid book with J. R. Norman, *Giant Fishes, Whales and Dolphins* are meticulously accurate.

A substantial work was that with P. E. Purves on Hearing in Cetaceans (*Bull. Brit. Mus. (Nat. Hist.) Zool.* 1960, 7, 1). This dealt with anatomical and experimental evidence to demonstrate that hearing was by way of the external auditory meatus, it was precisely discriminative and directional and the cetacean ear was sensitive to a wide range of frequencies. The elaborate sinus system was shown to be an extension of the middle ear cavity and could be a guide to the systematic arrangement of the Order Cetacea.

Fraser had a deep interest in the history of cetology and wrote about Peter Mundy's Greenwich Whale of 1568, early Japanese whaling, William Scoresby Junior and on the Grey Whale in Icelandic waters. His last contribution (1977) was on 'Royal Fishes: The Importance of the Dolphin'. This paper contained a lifetime's research and reflection on the Royal Prerogative in Britain which he had traced back as far as the 13th century.

Fraser was proud of his Dingwall stock and delighted in being a pawky Scot of the dry, humorous kind: yet there was nothing but solicitude and generosity about him, consideration for anyone deserving of it and an unrelenting determination to see justice done. It could well have been of him that Herman Melville wrote: 'But I have swam through libraries and sailed through oceans; I have had to do with whales with these visible hands; I am in earnest; and I will try.'

R. J. Harrison

A. S. McFarlane

ARTHUR SPROUL MCFARLANE, Head of the Biophysics Division at the National Institute for Medical Research from 1945 to 1970 died on 4 October 1978.

He was born in Glasgow on 18 April 1905. He took 1st class honours in biochemistry at Glasgow University in 1928, and qualified in medicine in 1931. From 1931–33 he was biochemist at the Glasgow Royal Cancer Hospital, and was elected to a Beit Memorial Fellowship in 1933 to work in Uppsala under Professor The Svedberg on the use of the recently developed oil-turbine ultracentrifuge. The studies which he made of a wide range of serum proteins illustrated the potential of this new technique for the characterisation of normal and pathological proteins in mixtures.

When the Rockefeller Foundation, anxious to make the instrument available to other laboratories, offered one to the Lister Institute, McFarlane joined the Institute to continue his Beit Fellowship and to supervise the installation of the Svedberg machine in a specially constructed building. The installation was completed in 1937, by which time he had become a member of the Lister Institute staff and had been joined by R. A. Kekwick. They also acquired the newly developed Tiselius electrophoresis apparatus—both instruments being the first of their kind in the UK—and in 1938 published the first paper arising from their use on the 'Physical properties of bushy stunt virus' recently crystallised by F. C. Bawden and N. W. Pirie.

Further work on vaccinia virus was interrupted by the war, when his attention changed to devising a means for preparing stable human plasma for transfusion. He invented a novel means of removing unstable lipids by freezing below -25°C with ether, and went on to design large scale freeze drying plant. This was done at the L.C.C. Serum Institute, Carshalton, where the laboratory had been transferred in 1941.

In 1943 McFarlane's services were requested by the National Institute for Medical Research to supervise the design and installation of special services, including one of the early electron microscopes, in the new building at Mill Hill (built in 1938 but used meanwhile by units of the Women's Royal Naval Service and finally occupied in 1950).

When in 1945 the US Atomic Energy Commission announced that radioisotopes produced in uranium chain-reactors would be available for peaceful uses to scientists all over the world, McFarlane was sent by the Medical Research Council to negotiate the supply of radioisotopes for the UK.

Hopes were rudely shattered by the passage in 1946 of the McMahon Act forbidding exportation from the US of all radioactive materials produced in atomic piles, but McFarlane through his friendship with Robley Evans, in charge of the cyclotron at the Massachusetts Institute of Technology, managed to obtain sufficient ^{32}P to allow research workers here to get the feel of radioisotopes and experience in design of counting instruments.

In 1947 he was again sent to the US and to Canada to negotiate purchase of other radioisotopes and this mission was entirely successful. (A fuller account appears in an article by Professor George Popjak in *Trends in Biochemical Sciences*, October 1976.)

By the time that the N.I.M.R. finally moved to Mill Hill in 1950 McFarlane had been responsible for making it the best equipped medical research laboratory in England in respect of biophysical instrumentation. By designing an elaborate but efficient apparatus for counting ^{14}C after combustion to $^{14}\text{CO}_2$ and installing a mass spectrometer he also gave his colleagues a headstart in the use of the radioactive and stable isotopes then available.

His own interests became concentrated on studying the metabolism of plasma proteins, using ^{14}C and radioiodine as trace labels. He devised a method for labelling proteins with radioiodine, which avoided denaturation and by studies on antibodies in collaboration with J. H. Humphrey showed that biological activity and ^{14}C internal and ^{131}I external labels were catabolised at identical rates. This validated radioiodine labelling and led to a series of definitive measurements on the turnover of several plasma proteins in normal and sick subjects, in which McFarlane collaborated with workers in Britain, the USA and elsewhere. He also developed an ingenious method for measuring plasma protein synthesis *in vivo* involving simultaneous measurement of incorporation of ^{13}C from labelled urea and ^{14}C from bicarbonate.

By temperament he was a shrewd Scotsman, with what might nowadays be considered an old fashioned outlook on life, but certainly capable of enjoying it. He was at his best when devising apparatus or techniques, and would have made his mark in industry had he chosen this career. Although his own approach to biological problems was simple (sometimes over-simple) and mathematical, he greatly helped many biological colleagues by providing them with technological back-up at a time when the array of sophisticated equipment nowadays provided by scientific instrument manufacturers was not available.

J. H. Humphrey

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